Sara Alexandra Anacleto Mateus

Bachelor Degree in Biomedical Sciences

From fruit pulp wastes to biomethane: assessment of substrate shifts on the performance of a two-stage anaerobic system and biogas upgrading studies

Dissertation to obtain Master Degree in Biotechnology

Supervisor: Joana Cassidy, Post-doctoral researcher, FCT/UNL

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Abstract

Wastes with high organic content, such as food waste, are produced worldwide and can cause serious pollution problems when poorly managed. Thus, there is the need for the implementation of environmental friendly treatment systems for organic wastes. Anaerobic digestion has the potential to contribute for the sustainable treatment of these wastes while producing biogas which provides a renewable energy source, methane (CH_4).

In this study, a two-stage anaerobic system was operated treating three different fruit pulp wastes (peach, raspberry and white guava) in a sequential operation. The effect of substrate shifts and different operational conditions, such as hydraulic retention time (HRT), organic loading rate (OLR) and pH on the system's performance was assessed. The shift of substrates caused no long-term instability issues. The differences observed in the acidogenic performance in terms of gas production between substrates were considerable. Conversely, only slight differences were observed in fermentation products (FP) concentration and profiles. No evident association was found between pH and HRT/OLR changes on FP concentration and profiles in the range studied. Overall, the sugar removal efficiencies obtained were between 93.8 – 97.8% and the acidification degree varied between 53.7% – 76.4%. In regard to the methanogenic reactor, biogas production (3.6 – 12.8 L d^{-1}) increased as OLR increased up to 7.4 g COD L^{-1}, while CH_4 yield (0.30 – 0.37 L CH_4 g^{-1} COD) and content (75.9 – 80.6%) remained approximately constant. Maximal chemical oxygen demand (COD) removal efficiency (around 93%) was achieved at HRTs of 8.6 and 5 days (OLR of 1.9 – 3.7 g COD L^{-1} d^{-1}).

Currently, there is the need to develop effective and economical viable solutions for biogas upgrading. Thus, gas permeation studies using mixed-matrix membranes (MMMs) with two different metal organic frameworks (MOFs) - MIL-53 and MOF-5 - were carried out in other to assess the potential for CH_4 and carbon dioxide (CO_2) separation. Matrimid®5218 with 10% (w/w) MIL-53 membrane showed the best performance among the membranes tested.

Keywords: two-stage anaerobic digestion system; fruit pulp wastes; substrate shifts; biomethane; biogas upgrading; mixed-matrix membranes

Resumo

Os resíduos ricos em matéria orgânica, como resíduos alimentares, são constantemente produzidos mundialmente e podem causar graves problemas de poluição se não forem devidamente tratados. Assim, existe a necessidade de implementar sistemas de tratamento viáveis do ponto de vista ambiental e económico para os resíduos orgânicos. A digestão anaeróbia pode contribuir para o tratamento sustentável destes resíduos, fornecendo uma fonte de energia renovável, o metano, através da produção de biogás.

Neste estudo, três resíduos de polpa de fruta (pêssego, framboesa e goiaba branca) foram sequencialmente tratados num sistema de digestão anaeróbia de duas fases. Foi avaliado o efeito de mudanças de substrato e de condições operacionais como o tempo hidráulico de retenção, a carga orgânica e pH no desempenho do sistema. A mudança de substrato não causou instabilidade no sistema a longo prazo. Em relação ao reator acidogénico, foram detetadas diferenças consideráveis na produção de gás entre substratos. No entanto, apenas foram detetas diferenças ligeiras na produção e perfil dos produtos de fermentação. Na gama estudada, não foi encontrada uma associação evidente entre o pH ou o TRH/carga orgânica e a produção e perfil dos produtos de fermentação. Foram obtidas remoções de açúcar entre 93.8 – 97.8% e graus de acidificação entre 53.7% – 76.4%. Em relação ao reator metanogénico, a produção de biogás (3.6 – 12.8 L d^{-1}) aumentou com o aumento da carga orgânica até 7.4 g CQO L^{-1} enquanto que o rendimento em metano (0.30 – 0.37 L CH_4 g^{-1} CQO) e a sua percentagem se manteve aproximadamente constante (75.9 – 80.6%). A remoção de carência química de oxigénio (CQO) foi máxima (aproximadamente 93%) operando a TRHs de 8.6 e 5 dias (carga orgânica de 1.9 – 3.7 g CQO L^{-1} d^{-1}).

Atualmente, existe a necessidade de desenvolver soluções eficazes e economicamente viáveis para o processo de purificação de biogás. Como tal, estudos de permeação gasosa usando membranas de matriz mista com diferentes *metal organic frameworks* (MIL-53 e MOF-5) foram conduzidos de forma a avaliar o seu potencial para a separação de metano e dióxido de carbono. A membrana Matrimid®5218 com 10% (m/m) MIL-53 foi a que demostrou um desempenho superior.

Palavras-chave: Sistema de digestão anaeróbia de duas fases; resíduos de polpa de fruta; mudança de substrato; biometano; purificação de biogás; membranas de matriz mista

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List of Abbreviations

$\alpha CO_2/CH_4$	Ideal selectivity of CO_2 over CH_4
AD	Anaerobic digestion
COD	Chemical oxygen demand
CSTR	Continuous stirred-tank reactor
EtOH	Ethanol
FISH	Fluorescence in situ hybridisation
FP	Fermentation products
FVW	Fruit and vegetable waste
FW	Food waste
GC	Gas chromatography
HAc	Acetic acid
HBut	Butyric acid
HLac	Lactic acid
HOrg	Organic acid
HRT	Hydraulic retention time
HPr	Propionic acid
HVal	Valeric acid
MMM	Mixed matrix membrane
MOF	Metal organic framework
OLR	Organic loading rate
PCO_2	CO_2 permeability
SCOD	Soluble chemical oxygen demand
TCOD	Total chemical oxygen demand
TS	Total solids
TSS	Total suspended solids
VFA	Volatile fatty acid
VS	Volatile solids
VSS	Volatile suspended solids

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Introduction

1.1 Problem statement

Population growth and subsequent industrialisation leads to the generation of enormous amounts of different types of waste which contribute to water and air pollution when unduly treated and discharged (Chan et al., 2009). According to Eurostat, 891 million tonnes of waste, excluding mineral waste, were generated in the European Union during 2014 (Eurostat, 2017). These wastes originated from diverse sectors, such as water and waste services, households and manufacturing activities. Conventional treatments such as incineration or landfills lead to the emission of greenhouse gases (GHG) and to the production of leachate (in the case of landfills) (Jiang et al., 2013 and references therein; Li et al., 2017 and references therein; Sen et al., 2016). On the other hand, most wastewaters are treated in centralised plants which implies not only transportation costs but also the consumption of water and energy (Paudel et al., 2017). The waste and wastewater generated from food industries, including fruit juice industries, usually have low pH and contain high amounts of organic matter, dissolved and suspended solids, oil and grease (El-Kamah et al., 2010; Ozbas et al., 2006). Hence, the generated waste needs appropriate treatment in order to meet the legislation for discharge in water or for reuse for agricultural application (El-Kamah et al., 2010). In this sense, taking into account the problems associated with current treatment strategies, there is a need to develop economic and environmental sustainable treatment technologies to be applied in industry facilities. The need to implement these treatment processes has been recognised since 1997 when incentives were created under the Kyoto Protocol to reward the companies that develop and use on-site treatment systems (Chan et al., 2009).

In this context, the importance of circular economy and biorefineries is increasing. Circular economy is slowly substituting the present linear economic model of *"take-make-consume-dispose"* with a sustainable approach which is meant to maintain and retain the value of materials and products (EEA, 2016). Thus, an integrated system for the treatment of wastewaters and other wastes with resources recovery or production is an essential contribution for a global sustainable development (Batstone and Virdis, 2014; Puyol et al., 2017). Examples of integrated systems include the production of biopolymers, the recovery of nutrients such as nitrogen and phosphorus and the generation of bioenergy as biohydrogen and biogas through photo and dark fermentation and anaerobic digestion (AD), respectively (Puyol et al., 2017).

Among the examples referred above, biogas production can contribute to overcome the current energy crisis. Presently, energy demands are met relying mostly on exhaustible fossil fuels' combustion which releases GHG, such as carbon dioxide (CO_2), thus actively contributing to air pollution and global warming (Zhou et al., 2017). The urgency for the implementation of low carbon-technologies for renewable energy production is recognised by government bodies worldwide even when the price of fossil fuels decreases (IEA, 2016). The strategy of Europe 2020 imposes that 20% of energy supply in Europe will rely in renewable energy sources by 2020 (Eurostat, 2016). Holm-Nielsen et al., (2007) anticipated that biogas produced from organic material is expected to conquer its place as a renewable energy resource representing at least 25% of all bioenergy produced. In fact, bioliquids and biogas represented 15% of the total gross inland consumption of renewable energy in

the EU in 2014 showing an important increase when comparing to the 3% obtained in 2000 (Eurostat, 2016).

Taking into account the problems exposed, the present work aimed at achieving the concomitant treatment of fruit juice industry wastes and the production of biogas through AD at laboratory scale. Furthermore, as the removal of CO_2 from biogas is an essential and current limiting step for its commercial application, the development of membranes for biogas upgrading was also performed. When applied at industrial scale, this AD system would enable the on-site treatment of wastes reducing the amount of wastes discarded to municipal treatment facilities and related costs. Moreover, the biogas produced could be directly used on-site to provide energy for the AD system itself, for heating and electricity or as vehicle fuel and natural gas after upgrading.

1.2 Anaerobic digestion process

AD is a complex synergetic biological process that occurs in the absence of oxygen. In AD, a consortium of facultative and strict anaerobic microorganisms converts organic matter into biogas (Chiu and Lo, 2016; Tauseef et al., 2013). Biogas is mainly composed of methane (CH_4) (60 – 70 %) and CO_2 (30 – 40 %), with trace amounts of hydrogen sulphide (H_2S), ammonia (NH_3), hydrogen (H_2), nitrogen (N_2) and carbon monoxide (CO) (Sun et al., 2015). AD occurs naturally in environments such as watercourses, swamps and ponds (Christy et al., 2014). However, human activities as petroleum and natural gas production, landfills, animal husbandry and waste management are responsible for around 90% of global CH_4 emissions (Zhou et al., 2017 and references therein). The uncontrolled release of CH_4 constitutes an environmental concern since it is a GHG whose greenhouse warming potential (GWP) is 23 times higher than CO_2 (van Lier, 2008). However, its controlled production provides a clean renewable energy source capable of replacing the ones derived from fossil fuels, contributing for the control of GHG emissions and ultimately global warming reduction (Chynoweth et al., 2001). After biogas upgrading and cleaning, biomethane has the potential to be directly used as fuel for combustion engines, gas turbines and fuel cells. It can also replace natural gas or be used as vehicle fuel (Zhou et al., 2017). Furthermore, the effluent of anaerobic methane-producing processes can be valorised since it can be used as an organic solid fertilizer (Demirel and Scherer, 2008).

AD has a higher treatment performance when compared to conventional biological treatments, such as aerobic processes (e.g. activated sludge) and lagoons when treating high strength wastewaters (> 4 g COD L^{-1}) (Hamza et al., 2016). The use of AD in the treatment of the latter presents advantages, such as lower production of sludge, lower energy and nutrient requirements, the production of bioenergy and the potential for posterior nutrient recovery. Nevertheless, it is difficult to completely remove all organic matter. Thus, depending on the final destination of the effluent, a post-treatment (e.g. aerobic treatment) may be necessary in order to obtain a higher quality effluent which meets the discharge standard (Chan et al., 2009). Several industrial and municipal organic wastes and wastewaters have been successively treated by AD. Examples include cheese whey (Diamantis et al., 2014; Yilmazer and Yenigün, 1999), food waste (FW) (Ariunbaatar et al., 2015; Chu et al., 2008), fruit juice waste (Ozbas et al., 2006), fruit and vegetable waste (FVW) (Ganesh et al., 2014; Mtz-Viturtia et

al., 1995; Wu et al., 2016) waste vegetable oil and pig manure (Hidalgo et al., 2014), dairy (Ince, 1998) and agricultural residues (Parawira et al., 2008).

1.2.1 Microbiological aspects and main pathways of anaerobic digestion

The AD process involves four main steps, where organic matter is converted to biogas: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Figure 1.1). The inherent biochemical transformations occur due to the presence of different groups of microorganisms (Table 1.1).

During hydrolysis, complex polymers are digested into soluble molecules by hydrolytic exoenzymes (such as cellulases, lipases, proteases) which are secreted by hydrolytic bacteria (van Lier et al., 2008). Degradation of recalcitrant substances namely cellulose and lignin is relatively slow, possibly being a rate limiting step (Amani et al., 2010). However, when the substrates have low cellulose content such as in FVW, methanogenesis is the rate limiting step (Bouallagui et al., 2005). Acidogenic bacteria convert the hydrolysis products in various organic acids (HOrgs) (e.g. lactic, acetic, butyric, propionic and valeric acids), alcohols, CO_2 and H_2. Acidogenesis is usually the fastest stage of AD as acidogenic microorganisms present thirty to fortyfold higher growth rates when compared to methanogenic microorganisms. Moreover, acidogens are able to resist extreme conditions like high temperature, low pH and high organic loading rates (OLRs) (Amani et al., 2010).

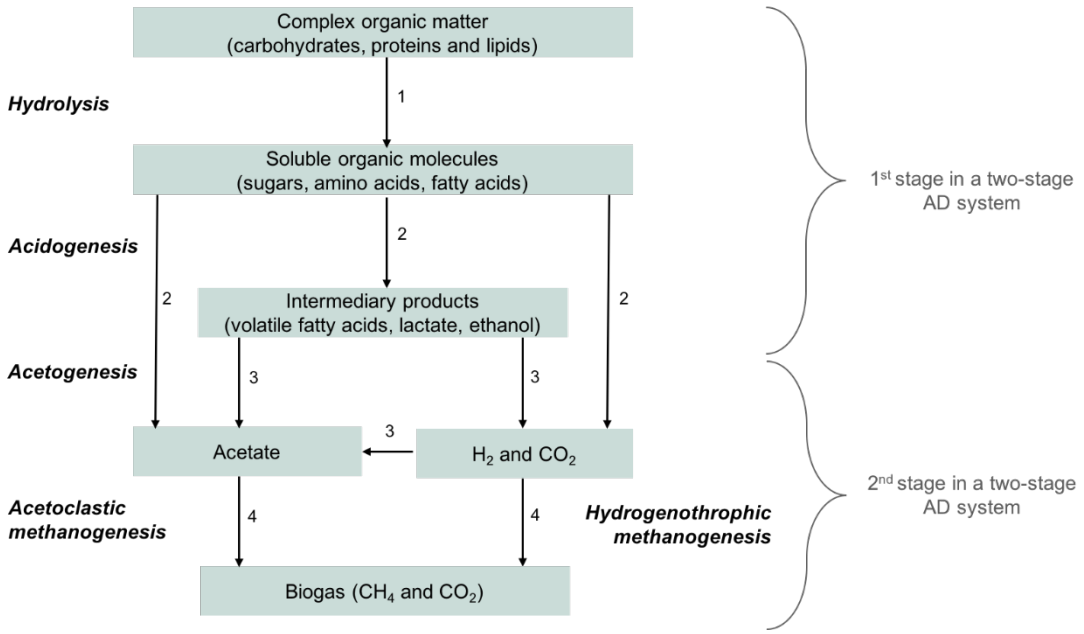

Figure 1.1 Main stages and the microorganisms involved in the anaerobic digestion process. 1. Hydrolytic bacteria; 2. Acidogenic bacteria; 3. Acetogenic bacteria; 4. Methanogens (Adapted from Cassidy, 2014 and Gerardi, 2003)

The third step of AD, acetogenesis, is characterised by the conversion of organic acids and alcohols into acetate, H_2 and CO_2 (Cazier et al., 2015). Between 25 – 55ºC, the free energy associated with the conversion of propionate and butyrate to acetate and H_2 is positive ($\Delta G > 0$), i.e., these conversions are unfavourable reactions. Low H_2 concentration, *i.e,* less than 10^{-4} atm and 10^{-5} atm is required for the conversion of butyrate and propionate, respectively (Amani et al., 2010). The low H_2 partial

pressure is maintained by the syntrophic relationships between H_2-producers and H_2-consumers, termed as *interspecies hydrogen transfer,* where hydrogenotrophic methanogens consume the H_2 produced by acidogens and acetogens (Metcalf&Eddy, 2003 and references therein).

Hydrogenotrophic and acetoclastic methanogens are the two main groups of methanogenic archaea responsible for the production of CH_4 (and CO_2, H_2 and other residual gases) during methanogenesis (Gonzalez-Martinez et al., 2016; Goswami et al., 2016). Hydrogenotrophic methanogens produce CH_4 using H_2 as the electron donor and CO_2 as electron acceptor, while acetoclastic methanogens convert acetate into CH_4 through decarboxylation (Metcalf & Eddy, 2003). Acetate degradation is usually responsible for 65% to 95% of CH_4 production when there is a limited supply of H_2 in the reactor (Amani et al., 2010 and references therein).

Table 1.1 Examples of microorganisms responsible for each stage of AD (Christy et al., 2014; Divya et al., 2015)

Stage	Microorganism
Hydrolysis	Clostridia, Micrococci, *Bacteroides,* Bacilli
Acidogenesis	Clostridia, *Flavobacterium,* Proteobacteria, *Pseudomonas,* Bacilli, Gammaproteobacteria
Acetogenesis	Clostridia, *Syntrophomonas, Syntrophobacter, Syntrophospora*
Methanogenesis	Methanobacteria, *Methanobacillus,* Methanococcus, *Methanosarcina*

1.2.2 Two–stage anaerobic digestion system

AD is typically conducted in a single reactor system. However, the microorganisms involved in AD are distinct in terms of physiology, growth kinetics, nutritional needs and sensibility to environmental conditions (Demirel and Yenigün, 2002). Thus, in a single reactor, the microorganisms are not subjected to their growth and activity optimum conditions (temperature, pH, OLR), which can cause stability and control issues throughout the operation (Khan et al., 2016). In order to provide the optimal conditions for each group of microorganisms, a two-stage anaerobic digestion system was proposed by Pohland and Ghosh (1979) in which the hydrolysis and acidogenesis stages are physically separated from the methanogenesis stage using two reactors (Figure 1.1) (Lindner et al., 2016 and references therein).

In a two-stage AD system, organic matter is hydrolysed and converted to HOrgs in the first reactor by hydrolytic and acidogenic bacteria while methanogenic archaea produce CH_4 from acetate (converted from HOrgs by acetogenic bacteria) and H_2 in the second reactor (Gonzalez-Martinez et al., 2016). Both reactors can be inoculated with the same anaerobic biomass, coming from one-stage AD systems. However, as each reactor is operated in specific conditions for the microbial community of interest, there is an enrichment of each community in its respective stage (Solera et al., 2002). For instance, the low pH and shorter hydraulic retention time (HRT) and, consequently, higher OLR normally imposed in the acidogenic reactor prevents methanogens' survival in this stage. Likewise, as the substrate for acidogens is almost completely consumed in the acidogenic reactor, these bacteria are not able to thrive in the methanogenic reactor (Dareioti et al., 2009).

In a single reactor, the increase of OLR causes kinetic imbalances since methanogens grow considerably slower than acidogens (Ghosh et al., 1985). The fast production of volatile fatty acids (VFAs) by acidogens can result in the accumulation of VFAs at inhibitory levels for methanogens also leading to pH decrease and ultimately to process failure (Xiao et al., 2013; Zuo et al., 2015). In two-stage systems, the first phase works as a buffer of varying OLRs providing a more homogeneous influent for the second reactor (Demirel and Yenigün, 2002; Voelklein et al., 2016; Xiao et al., 2013). This configuration allows the treatment of higher OLRs, achieving higher COD removal efficiencies and CH_4 production (Khan et al., 2016; Shen et al., 2013; Xiao et al., 2013). Although two-stage systems involve higher initial building and maintenance costs, this configuration is still economically competitive with single stage systems (Ghosh et al., 1985; Ward et al., 2008). Two-stage systems are especially advantageous when treating easily hydrolysable substrates with a high sugar content and overall wastes with high organic content such FW (Grimberg et al., 2015; Lindner et al., 2016).

The separation of the acidogenic and methanogenic phase results in different contents of CO_2, H_2 and CH_4 in each phase. CO_2 and H_2 are the main gases produced in the first stage whereas CH_4 production is minimised and in some cases not produced. The production of CO_2 in the acidogenic phase leads to the achievement of higher CH_4 content in the biogas produced in the methanogenic reactor, compared to single stage systems. Consequently, the costs and energy demand of the upgrading system for CO_2 removal in two-stage systems is lower when compared to single-stage systems, which is an important advantage since biogas upgrading can represent up to 30% of the costs of the whole system (Voelklein et al., 2016 and references there in).

Two-stage anaerobic systems have been successfully applied in the treatment of several wastes such as municipal solid wastes, agroindustrial and food residues (Dareioti et al., 2009 and references there in). Fu et al., (2017) compared the performance of single stage vs two-stage systems treating vinasses, an easily degradable waste. The two-stage operation presented a higher performance in terms of CH_4 yield (10.8% higher), volatile solids (VS) removal efficiency (10.4% higher) and energy recovery (12.9% higher). Moreover, the lag-phase was 9.1 days shorter in the two-stage system than in the one stage system. In another study, treating synthetic FW, the two-stage system showed higher tolerance to organic loading shocks and higher CH_4 production than the one stage system (Ariunbaatar et al., 2015). Overall, two-stage configurations achieved superior performances in studies comparing single and two-stage systems operating in similar conditions, both at laboratorial and pilot scales (Ariunbaatar et al., 2015; Aslanzadeh et al., 2014; Grimberg et al., 2015; Fu et al., 2017; Hidalgo et al., 2014; Leite et al., 2016; Luo et al., 2011; Massanet-Nicolau et al., 2015; Nathao et al., 2014; Voelklein et al., 2016).

1.2.3 Environmental and operational conditions

The success of AD depends on several factors, such as the reactor configuration, mixing strategy and biomass growth systems. Furthermore, in order to assure process stability, some key parameters must be carefully controlled since microorganisms, especially methanogens, are very susceptible to environmental changes. These parameters include temperature, pH, nutrients, HRT and OLR.

1.2.3.1 Reactor configuration and mixing

AD systems can be categorised into “low rate” and “high rate” systems. Low rate systems are operated with long HRTs and are mainly used for the treatment of slurries and solid waste. Examples of this type of system include batch operation, accumulation, plug flow and continuous stirred-tank reactor (CSTR). High rate systems are operated at shorter HRTs including contact process, anaerobic filter, fluidised bed, upflow anaerobic sludge blanket (UASB) and expanded granular sludge bed (EGSB), which are mainly applied in wastewater treatment (De Mes et al., 2003). A key factor for high rate AD systems success is the separation of the HRT from the solids retention time (SRT) accomplished through sludge retention (e.g. sedimentation, granulation) or the separation of bacterial sludge from the effluent for posterior recirculation to the reactor (Van Lier, 2008).

In order to facilitate the transfer of organic material and its contact with the active biomass, a mixing strategy, either continuous or intermittent, is advisable. Mixing also promotes the release of gas bubbles trapped in the broth and prevents sedimentation of particulate material (Ward et al., 2008). Mixing can be accomplished by mechanical mixers or by recirculation of the digestate or the produced gas (Rittmann and McCarty, 2001; Ward et al., 2008).

1.2.3.2 Anaerobic granular biomass

AD can be promoted in suspended growth systems or attached growth systems. In suspended growth systems, the microorganisms are suspended in the liquid (as flocs or granules) through mixing. In the attached growth systems, the microorganisms are attached to an inert material forming a biofilm (Metcalf&Eddy, 2003; van Lier, 2008). Granular sludge is associated to higher biomass retention times and resistance to higher OLRs and toxic shocks (Amani et al., 2010). Park et al (2016) also stated advantages of granular biomass concerning lag time and CH_4 yield due to the close proximity of microorganisms in the granule which improves the substrate mass transfer. Granules are densely aggregated structures formed by the self-immobilisation of microorganisms from different species organised in layers (Park et al., 2016). Some authors defend that acetoclastic methanogens are mainly situated in the inner layer of the anaerobic granules being protected from toxic materials, such as free ammonia and sulphide and also from high H_2 concentrations. The second layer includes H_2-producing acetogens and H_2-consuming methanogens and the outer layer contains fermentative bacteria (Fang et al., 1994; Lim and Kim, 2014; Macleod et al., 1990; Park et al., 2016). The close proximity of H_2-producing and H_2-consuming microorganisms contributes for an efficient propionate and butyrate conversion (see section 1.2.1), avoiding the accumulation of VFA (Amani et al., 2010 and references therein). The addition of certain ions in specific concentrations (e.g calcium, magnesium,

aluminium and ferrous ions) has been proved to improve granulation rates due to the reduction of electrostatic repulsion between negatively charged microorganisms (Amani et al., 2010).

1.2.3.3 Temperature

AD can be operated at mesophilic (35 – 40 ºC) or thermophilic (55 – 60 ºC) temperatures (Rittmann and McCarty, 2001). Temperature influences the enzymes' activity, CH_4 yield and effluent quality. Slight variations as small as 1 ºC/day can lead to process failure in thermophilic operations (Zhang et al., 2014). On the other hand, temperature changes of +/- 3 ºC have no significant impact on the CH_4 production under mesophilic operation (Weiland, 2010). Although thermophilic AD is associated to higher metabolic rates and CH_4 productivities, it also requires higher investments and net energy input, produces effluents with lower quality and is more susceptible to environmental conditions when compared with mesophilic AD (Mao et al., 2015).

1.2.3.4 pH and alkalinity

Microorganisms' growth rates are greatly affected by pH and the optimal values for acidogens and methanogens are very different. This fact constitutes a main driver for the implementation of two-stage systems. In this configuration, the first reactor is usually operated at a pH of 4 – 6 favouring hydrolysis and acidification while the second reactor is operated at a neutral pH favouring methanogenesis (Voelklein et al., 2016 and references therein). As methanogenesis is considered a rate-limiting step and methanogens are more susceptible to pH changes, a neutral pH (7 – 8) is chosen for single stage operations (Weiland, 2010). At high pH values (> 8), methanogens' activity can be inhibited due to ammonia toxicity, while at lower pH values, acidogenesis will prevail possibly leading to VFA accumulation which can result in a further pH drop (Khanal, 2008). Nonetheless, a high alkalinity, *i.e.* buffering capacity is able to protect the system against these rapid pH drops and depends on the equilibrium of gaseous CO_2 and bicarbonate ions (Ward et al., 2008). In a properly operating reactor, alkalinity should vary between 2000 and 4000 mg L^{-1} of $CaCO_3$ (APHA, 1999). Alkalinity may be self-generated by the system (natural alkalinity) through the degradation of organic matter, mainly when treating wastes rich in organic nitrogen (e.g. protein). Alternatively, the addition of chemicals (e.g. sodium bicarbonate, hydroxide-based chemicals) contributes for the increase of alkalinity (Khanal, 2008).

1.2.3.5 Nutrients

Nutrient balance, commonly evaluated by C:N:P (carbon:nitrogen:phosphorus) ratio, is another important parameter since it affects biomass growth and consequently, the stability of the operation. The theoretical ratio may be calculated considering the empirical formula of anaerobic biomass. Nevertheless, the optimum C:N:P ratio also depends on the substrate and inoculum (Khanal, 2008; Zhang et al., 2014). Nitrogen and phosphorus may need to be added when wastewaters present low nutrient content (Metcalf&Eddy, 2003). Besides these macronutrients, several micronutrients (trace minerals) such as iron, calcium, magnesium, selenium and cobalt, among others, are also necessary for the growth of microorganisms (Khanal, 2008).

1.2.3.6 HRT and OLR

HRT corresponds to the time that a soluble component remains in the reactor and should be optimised in order to allow the complete degradation of the organic matter. This parameter is mathematically defined by the equation HRT = V/Q, where V is the reactor volume and Q represents the influent flow rate (Abdelgadir et al., 2014; Mao et al., 2015). The optimal HRT value depends on influent composition, process details and temperature (Amani et al., 2010). OLR depends on the HRT and on the chemical oxygen demand (COD) concentration of the substrate (Paudel et al., 2017) and is commonly considered as the COD concentration of the influent entering the reactor per day under continuous feeding (Mao et al., 2015). Fluctuations in loading rates can negatively impact the balance between acidogenesis and methanogenesis in single stage reactors. Acidogens are able to operate at high loading rates producing an amount of VFAs which methanogens may not be able to consume given their slower growth rates (Metcalf&Eddy, 2003). A low OLR and high HRT may be a safe strategy to obtain constant and maximal CH_4 yields (Mao et al., 2015). However, it is desirable to achieve a short HRT reducing the reactor volume and the capital costs which will allow the treatment of higher amounts of waste (Speece, 1983).

1.3 Biogas upgrading

Biogas must be cleaned and upgraded to biomethane in order to be used as natural gas or achieve vehicle fuel standards. For instance, it is necessary to obtain concentrations of CH_4 >80 – 96%, CO_2 <2 – 3%, O_2 <0.2 – 0.5%, H_2S <5 mg m^{-3}, NH_3 <3 – 20 mg m^{-3} to be used as vehicle fuel (Chaemchuen et al., 2016). Biogas purification aims the removal of trace compounds and biogas upgrading is focused on the separation of CH_4 and CO_2 in order to enhance the calorific value improving the combustion efficiency (Andriani et al., 2014; Ryckebosch et al., 2011). Although CH_4 is the compound of interest for energy production, CO_2 can also be used in industrial applications such as enhanced oil recovery and sodium bicarbonate production (Sun et al., 2015). Indeed, the revalorisation of CO_2 is also important to further reduce GHG emissions, since most of CO_2 is still released into the atmosphere. Hence, some biogenic carbon sequestration methods are being investigated, such as CO_2 enrichment of anaerobic processes for further CH_4 production (Alimahmoodi and Mulligan, 2008; Fernández et al., 2015) and CO_2 uptake by microalgae (Bahr et al., 2014; Meier et al., 2015; Yan et al., 2016).

Several upgrading technologies, such as physical and chemical CO_2-absorption, Pressure Swing Adsorption (PSA), cryogenic separation and membrane processes are used for CO_2 removal. The selection of the technology to be implemented relies not only on the highest achievable CH_4 content but also on economic and ecological aspects (Ryckebosch et al., 2011). In the present work, membrane-based processes are the main focus. The advantages of this technology include low capital costs, high energy efficiency, simple operation and maintenance without the use of hazardous chemicals or solvents and also the fact that the resulting gas is already at natural gas grid pressure (Makaruk et al., 2010; Scholz et al., 2013).

1.3.1 Membrane-based processes

Membrane-based gas separation processes are based on the faster selective permeation of certain gases present in a mixture through a barrier under an external driving force (e.g. partial pressure, concentration or chemical potential gradient). In biogas upgrading using membranes, CO_2 permeates through the membranes while CH_4 is retained. In this type of separations, contaminants such as water vapour and N_2 are also eliminated (Zhou et al., 2017). Membranes can be classified as polymeric (e.g. polyacetylenes, polyimides) or inorganic membranes (e.g. zeolite, sol-gel derived, zirconia). Although the latter usually offers superior selectivities and permeabilities, polymeric membranes are the most used industrially as they are cheaper, easier to produce, easily scalable and present an excellent mechanical stability (Andriani et al., 2014; Scholz et al., 2013; Zhou et al., 2017). The main drawback of polymeric membranes is the trade-off between permeability and selectivity, as shown by Robeson (2008). In order to improve the characteristics of polymeric membranes, mixed matrix membranes (MMMs) are currently being investigated and are still not available commercially (Scholz et al., 2013). In MMMs, inorganic fillers are dispersed in the form of micro/nano-particles in a polymeric matrix (Zhou et al., 2017). In this way, MMMs combine the ease of fabrication of polymeric membranes and the superior transport properties of inorganic materials (Basu et al., 2011). Metal-organic frameworks (MOFs), which are crystalline and porous organic-inorganic hybrids formed by the coordination of metal ions with organic linkers, can be used as fillers for MMMs preparation (Chen et al., 2012 and references therein; Perez et al., 2009; Zhou et al., 2017). MOFs have large surface areas, controlled porosities and affinity for certain gases. Additionally, specific functional groups may be added to the MOF framework, optimising the pore size for extra affinity. MOFs have also showed promising results for size exclusion based gas adsorption (Dong et al., 2013 and references therein).

1.4 Scope of the thesis

This research focused on the operation of a two-stage anaerobic system at laboratorial scale treating different fruit pulp wastes from a juice industry in a sequential operation. As different fruits are harvested in specific seasons, it is almost impossible to operate the reactor for long periods of time using the same substrate. Therefore, it is important to study the effect of substrate shifts on the reactors performance and stability. Furthermore, gas permeation studies using MMMs were performed as preliminary steps for biogas upgrading.

The main objectives were: (1) To study the effect of different operational conditions (pH, OLR/HRT) on process performance (acidification degree, COD removal, biogas production and composition) and microbial communities' composition; (2) To investigate the effect of substrate shifts on process stability; (3) To assess the potential of MMMs with two different MOFs on CH_4 and CO_2 separation.

2

Materials and methods

2.1 Operation of a two-stage anaerobic digestion system

2.1.1 Substrate and inoculum

Fruit pulp waste (peach, raspberry and white guava) provided by Sumol+Compal Marcas S.A. (Almeirim, Portugal) was used as substrate in this study. Each fruit pulp waste was collected from the industry in a different season. The wastes were characterised (See Results and discussion section) and stored at -20 ºC until needed to prepare the influent. The influent was prepared three times per week diluting the fruit pulp waste with tap water (final concentration of 24 g COD L^{-1}). Nitrogen (as ammonium chloride, NH_4Cl) and phosphorus (as potassium di-hydrogen phosphate, KH_2PO_4) were supplemented to the influent at a mg COD: mg N: mg P ratio of 100:0.5:0.1 or 100:1:0.2, depending on the fruit pulp waste. The prepared influent was kept at 4 ºC with constant mixing.

The acidogenic reactor was inoculated with flocular anaerobic sludge (1:3) obtained from an acidogenic bioreactor treating a mixture of winery wastewater and grape concentrate (day 0). Due to the low biomass concentration observed, non-acclimatised anaerobic sludge from an anaerobic digester of a local municipal wastewater treatment plant (Beirolas - Sacavém, Portugal) was added to the reactor (1:3) on day 15. The methanogenic reactor was inoculated with a mixture of granular (1:6) and flocular sludge (1:6), since the granules were mainly constituted by hydrogenotrophic microorganisms, which can lead to acetate accumulation. The granular sludge was collected from a Biobed Expanded Granular Sludge Blanket (EGSB) reactor treating brewery wastewater from a beer industry and the flocular anaerobic sludge was collected from an anaerobic digester of Mutela wastewater treatment plant (Almada, Portugal).

2.1.2 Experimental setup and operation

A two-stage CSTR-CSTR system (CSTR-5S, Bioprocess Control, Sweden) was operated in continuous mode at lab-scale with 5 L of working volume in each reactor. The first reactor corresponded to the acidogenic phase and the second reactor corresponded to the methanogenic phase. The overall system configuration is presented in Figure 2.1. Control parameters such as pH, temperature, biogas flow rate and biogas volume were continuously monitored online (Bioprocess Control software). The CH_4 and CO_2 content in the biogas produced in the methanogenic phase was also monitored in real time (BenchOne Biogas, BlueSens, Germany). Both reactors were maintained at 30 ºC (mesophilic conditions) using a water bath system (CW-05G, Lab.companion, Jeio Tech, Korea). The pH was automatically controlled by the automatic addition of NaOH 5M.

The acidogenic and methanogenic reactors were stirred at 200 and 100 rpm, respectively. A settler was connected to each reactor promoting solids and biomass retention and recirculation and also the clarification of the effluent.

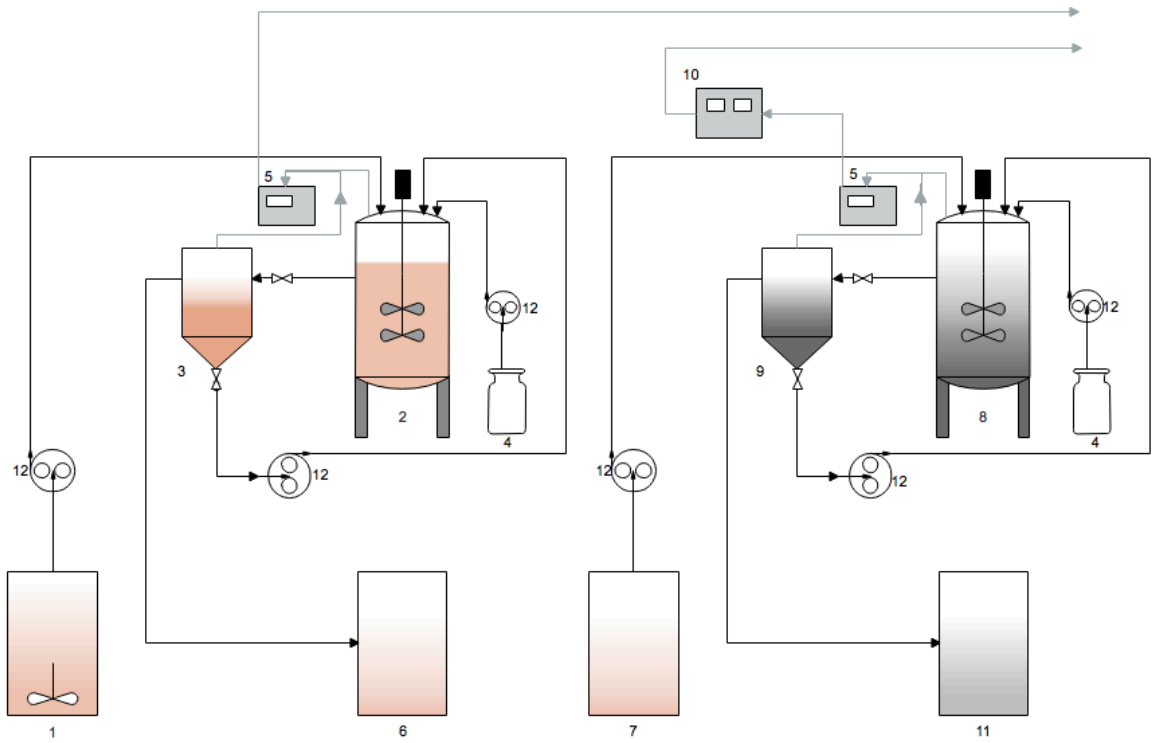

Figure 2.1 Schematic representation of the two-stage anaerobic system: (1) Acidogenic influent container; (2) Acidogenic reactor; (3) Acidogenic settler; (4) NaOH solution bolttle; (5) Gas flowmeter; (6) Acidogenic clarified fermentation broth container; (7) Methanogenic influent container (i.e. acidogenic clarified fermentation broth); (8) Methanogenic reactor; (9) Methanogenic settler; (10) Gas analyser (CH_4 and CO_2); (11) Methanogenic effluent container. Components not shown: computer for data acquisition; pH controller; water bath system.

The start-up conditions for the acidogenic reactor were pH of 5.5, HRT of 4 days and OLR of 7.0 ± 0.9 g COD L^{-1}. The reactor was later subjected to different conditions throughout a period of 285 days as indicated in Table 2.1. The methanogenic reactor was started after the acidogenic reactor produced a stable fermentation broth (107th day) (day 0 for the methanogenic reactor). The start-up conditions for the methanogenic reactor were pH of 7.5, HRT of 5 days, and OLR of 3.5 ± 0.1 g COD L^{-1}. However, due to fermentation products (FP) accumulation, the HRT was changed to 8.6 days on the 10th day of operation. The influent of the methanogenic reactor corresponded to the clarified fermentation broth of the acidogenic reactor, mainly containing the FP produced in the first stage. In order to promote granulation and granules' integrity, a solution of calcium chloride (10.5 mg of Ca^{2+} per gram of total suspended solids) was added to the methanogenic influent (Ismail, 2013). The conditions imposed on the reactor during the 178 days of operation are presented in Table 2.2. Every condition was maintained for at least 3 HRT.

Table 2.1 Operational conditions used in the acidogenic reactor

Substrate	Period	Time (d)	pH	HRT (d)	OLR (g COD L^{-1} d^{-1})
Peach pulp waste	I	0-26	5.5	4	7.0 ± 0.9
	II	27-35		2	11.9 ± 1.5
	III	36-51	4.5		11.9 ± 1.1
	IV	52-68	5.0		12.9 ± 2.4
	V	69-82		1	25.7 ± 0.9
	VI	83-110	5.5		24.1 ± 1.3
Raspberry pulp waste	VII	111-174			24.5 ± 4.2
	VIII	175-208	5.0		23.7 ± 3.1
	IX	209-234			11.9 ± 0.8
	X	235-256	4.5	2	11.6 ± 0.5
White guava pulp waste	XI	257-271			11.9 ± 0.9
	XII	272-285	5.0		10.7 ± 1.0

Table 2.2 Operational conditions used in the methanogenic reactor

Substrate	Period	Time (d)	pH	HRT (d)	OLR (g COD L^{-1} d^{-1})
Peach fermentation broth	I	0-10	7.5	5.0	3.5 ± 0.1
	II	11-41		8.6	1.9 ± 0.1
Raspberry fermentation broth	III	42-91			1.9 ± 0.1
	IV	92-135		5.0	3.7 ± 0.1
	V	136-165		2.5	7.4 ± 0.6
White guava fermentation broth	VI	166-178		2	6.8 ± 0.7

2.1.3 Analytical methods

Standard control parameters regarding AD experiments were monitored and quantified in order to assess reactor stability including the quantification of sugars, proteins, HOrgs and ethanol (EtOH), COD, ammonium nitrogen (NH_4-N), orthophosphate (PO_4-P), total suspended solids (TSS) and volatile suspended solids (VSS). Samples from both acidogenic and methanogenic influents, as well as from the reactors broth were collected two to three times per week. The samples were centrifuged at 11000 rpm for 2 minutes (Micro star 17, VWR, USA) and the supernatant was stored at -20 ºC until further analysis, except for acidogenic influent (only stored at -20ºC without centrifugation) and TSS and VSS samples (analysed in the same day). In order to compare results, sugars and FP concentrations were converted to g COD L^{-1} according to stoichiometry. To study the evolution of the microbial community in each reactor, samples for Fluorescence *in situ* hybridisation (FISH) analysis were collected in the first and last day of each condition tested.

2.1.3.1 Total and volatile solids and suspended solids

Total and volatile solids concentrations (TS and VS) were calculated for waste characterisation according to standard methods (APHA, 1999). In this analysis, TS was calculated after each well-mixed sample was placed in a weighed (Sartorius analytical scale) crucible and dried in the oven at 103 – 105 ºC overnight. For VS calculation, the crucible was further heated at 550 ºC for 2 hours and weighed again. TSS and VSS were also determined according to the standard methods (APHA, 1999) in order to monitor solids and biomass concentration. TSS and VSS analysis of the influents, reactors broth and clarified effluents was performed weekly and in duplicate. After weighing the glass fibre filters (1.2 μm, 47 mm), each well-mixed sample was filtered allowing the separation of TSS from total dissolved solids (TDS). After filtration, the filters were placed in aluminium dishes and dried at 103 – 105 ºC overnight. Filters were then weighed again for TSS determination and ignited at 550 ºC for 2 hours. At this temperature, the material that can be volatilised and burned off is mainly organic matter. Finally, a last weighing was performed and VSS concentration was determined. Due to the stratification of granular sludge in the second reactor, samples were collected at different heights, 0, 10, 17 and 25 cm from the bottom (h0, h1, h2 and h3, respectively). The average value for TSS and VSS in the methanogenic reactor was calculated following the trapezium rule.

2.1.3.2 COD

The Chemical Oxygen Demand (COD) measurement allows the quantification of the amount of organic matter present in the sample (De Mes et al., 2003). It takes into account the consumed amount of a certain oxidant when it reacts with the sample, being expressed in terms of its oxygen equivalence (APHA, 1999). COD concentration was measured by a colorimetric method using *Hach Lange* kits of the following concentration ranges: 5 – 60 g L^{-1} O_2 and 50 – 300 mg L^{-1} O_2. Sample preparation included filtration with a 0,2 μm syringe filter for soluble COD (SCOD) analysis and dilution according to the kit range. For total COD (TCOD) analysis, samples were not filtered. Samples were digested (Hach Lange HT 200S) at 170 ºC for 15 minutes. Finally, after cooling, COD concentration was measured using a spectrophotometer (Hach Lange DR 2800).

2.1.3.3 Organic acids and ethanol

Concentrations of acetic acid (HAc), butyric acid (HBut), lactic acid (HLac), valeric acid (HVal), propionic acid (HPr) and EtOH were determined by high performance liquid chromatography (HPLC), as described by Wang et al., (2017). A VWR Hitachi Chromaster chromatographer with an RI detector, a Biorad Aminex HPX-87H column (300 x 7.8 mm) and a Biorad pre-column (125-0129 30 x 4.6 mm) was used. The analysis was conducted at 30 ºC with sulphuric acid (H_2SO_4 0.005 M) as eluent at a 0.5 mL min^{-1} flow rate. HOrgs and EtOH concentrations were calculated using a standard calibration curve 31 – 1000 mg L^{-1} for each HOrg or EtOH. Sample preparation included the dilution of samples with H_2SO_4 (0.025 M) and filtration (0.2 μm pore size filter).

2.1.3.4 Sugars

Total sugar concentration was determined by Dubois method (Dubois et al., 1956), a colorimetric method based in a phenol-sulphuric acid reaction. A glucose solution was used as standard (3 – 100 mg L^{-1}) (results in equivalents of glucose). All samples, except the acidogenic influent, were filtered (0.2 μm pore size filter). All samples were diluted according their range of sugar content. Phenol 5% (0.5 mL) and H_2SO_4 98% (2.5 mL) were added to 0.5 mL of sample and then placed in the dark for 10 minutes. After this time, the samples were mixed in a vortex and placed again in the dark for 30 minutes after which the colour was stable. Finally, the absorbance was measured at 490 nm in the Hach Lange DR 2800 spectrophotometer.

2.1.3.5 Nutrients

The nutrients (NH_4-N and PO_4-P) concentrations were determined by a colorimetric method implemented in a continuous flow analyser (Skalar San ++, Skalar Analytical, The Netherlands), as described by Carvalheira et al., (2014). NH_4-N and PO_4-P concentrations were calculated using a standard calibration curve of 4 – 20 mg L^{-1} of N or P. Sample preparation included centrifugation of the samples and the dilution of the supernatant with Milli-Q water.

2.1.3.6 Proteins

Protein concentration was determined using a modified Lowry protein assay (Lowry et al., 1951). This colorimetric method is based on two main reactions: reaction of protein with copper in alkaline solution and the reduction of the phosphomolybdic-phosphotungstic reagent by the copper-treated protein. The reagents used were: solution A (10 g Na_2CO_3 + 0.1 g $C_4H_4KNaO_6.4H_2O$ + 500 mL NaOH 0.1 M); solution B (0.5 g $CuSO_4.5H_2O$ + 1 drop H_2SO_4 + 100 mL H_2O); solution C (solution A + solution B in a proportion of 50:1); solution D (Folin 50% (v/v)). A bovine serum albumin (BSA) solution was used as standard in the range of 6 – 200 mg L^{-1}. Firstly, 1.5 mL of solution C was added to 500 μL of the diluted samples followed by mixture in the vortex and incubation in the dark for 10 minutes at room temperature. Next, 150 μL of solution D was added, mixed in the vortex and incubated in the dark for 30 minutes at room temperature. Finally, the absorbance was measured at 750 nm in the Hach Lange DR 2800 spectrophotometer.

2.1.3.7 Gas analysis

Gas chromatography (GC) was performed to evaluate the composition of the gas produced in both reactors in terms of CH_4, CO_2, O_2, N_2 and H_2 content. The GC (Trace GC Ultra, ThermoFisher Scientific, USA) was equipped with a TCD detector and 30 meters of Carboxen 1010 Plot column. The mobile phase was helium with 1 mL min^{-1} of flow rate with isothermal runs during 50 minutes at 35° C. The injector temperature was 200°C.

2.1.4 Microbial community analysis by FISH

FISH was performed according to Amann (1995) aiming the identification of the microbial population in each reactor and possible variations associated with operational conditions' changes. In this method, specific labelled probes are applied in the biomass samples allowing the observation and identification of the communities through fluorescence microscopy. Fresh biomass samples were fixed with paraformaldehyde (4%) and stored at -20ºC. The fluorescently labelled oligonucletotide probes used were: Fluorescein isothiocyanate (FITC)-labelled EUB338mix (Bacteria), Cyanine 3 (Cy3)-labelled ARC915 (Archaea), ALF968 (Alphaproteobacteria), BET42a (Betaproteobacteria), GAM42a (Gammaproteobacteria), DELTA495a (Deltaproteobacteria), CF319a (Cytophaga, Flavobacteria), LGC0355 (Firmicutes), BAC303 (Bacteroidaceae, Prevotellaceae), MX825 (*Methanosaeta*), MB1174 (Methanobacteriales), MG1200b (Methanomicrobiales), MS821 (*Methanosarcina*). Details about each probe are found at probeBase (Greuter et al., 2016). Samples were observed using an epifluorescence microscope Imager D2 (Zeiss, Germany), at 1000X.

2.1.5 Calculations

To assess the acidogenic reactor performance, some parameters were calculated namely acidification degree, sugar and protein removal. Acidification degree may be calculated through Equation 1 where $\sum$[FP] is the sum of all FP concentrations in the reactor and [$TCOD_{in}$] is the total COD concentration in the influent or by Equation 2 where $\sum$[VFA] is the sum of all VFA concentrations. Sugar and protein removal was also determined following the general Equation 3 where X represents either sugar or protein.

$$\text{Acidification degree}_{FP} = \frac{\sum[FP]}{[TCOD_{in}]} \times 100, \text{ in \%} \qquad \text{(Equation 1)}$$

$$\text{Acidification degree}_{VFA} = \frac{\sum[VFA]}{[TCOD_{in}]} \times 100, \text{ in \%} \qquad \text{(Equation 2)}$$

$$\text{Removal}_{X} = \frac{[X_{in}] - [X_{out}]}{[X_{in}]} \times 100, \text{ in \%} \qquad \text{(Equation 3)}$$

COD removal, CH_4 productivity and CH_4 yield were determined in order to assess the performance of the methanogenic reactor. The removal of organic matter in the methanogenic phase was calculated using Equation 4 which relates the soluble COD in the fermentation broth ($SCOD_{in}$) and in the effluent ($SCOD_{out}$).

$$\text{COD removal} = \frac{[SCOD_{in}] - [SCOD_{out}]}{[SCOD_{in}]} \times 100, \text{ in \%} \qquad \text{(Equation 4)}$$

CH_4 productivity depends on the percentage of CH_4 in the produced biogas, the biogas flow rate and the reactor volume, as indicated in Equation 5. The CH_4 yield calculation (Equation 6) takes into account the same parameters as productivity but also the HRT and the difference between the SCOD in the fermentation broth and in the effluent (Δ[COD]). Finally, energy recovery was calculated considering the energy value of CH_4 as 37.38 kJ L^{-1} (Fu et al., 2017), as indicated in Equation 7.

$$CH_4 \text{ productivity} = \frac{\frac{\%CH_4}{100} \times \text{flow rate}}{V_{reactor}}, \text{ in L } CH_4 \text{ } L^{-1} d^{-1} \quad \text{(Equation 5)}$$

$$CH_4 \text{ yield} = \frac{\frac{\%CH_4}{100} \times \text{flow rate}}{\Delta[SCOD] \times V_{reactor}} \times HRT, \text{ in L } CH_4 \text{ } g^{-1} \text{ COD} \quad \text{(Equation 6)}$$

$$\text{Energy recovery} = CH_4 \text{ yield} \times 37.38, \text{ in kJ } g^{-1} \text{ COD} \quad \text{(Equation 7)}$$

2.2 Biogas upgrading using mixed matrix membranes with MOFs

2.2.1 MOF-5 synthesis and characterisation

MOF-5 ($Zn_4O(BDC)_3$) was synthesised according to Chen et al., (2010). Zinc nitrate hydrate, $Zn(NO_3)_2.xH_2O$ (Sigma-Aldrich, USA, purity 99.999%) was mixed with terephthalic acid, H_2BDC (Sigma-Aldrich, USA, purity 98%) in N,N-Dimethylformamide, DMF (Honeywell, Germany, purity ≥ 99.8%) and distilled water. The molar ratio of $Zn(NO_3)_2$: H_2BDC : DMF : H_2O was 1.0 : 0.76 : 175 : 12.8. The mixture was stirred to dissolve completely and transferred to Teflon-lined autoclaves which were heated at 120 ºC for 48h. After cooling to room temperature, the crystals were washed three times with DMF and dried at 150 ºC for 24h.

XRD patterns for MOF-5 characterisation were obtained using a MiniFlex II benchtop diffractometer (Rigaku Corportation, Japan) with CuKα radiation operating at 30 kV and 15 mA.

2.2.2 Membrane preparation

Mixed matrix membranes were prepared by the solvent evaporation method. Two MOFs were used as additives: MIL-53(Al) (Basolite® A100, Sigma-Aldrich) and MOF-5. Different additive loadings were tested namely 10%, 20% and 30 % (w/w). In general terms, a certain amount of Matrimid® 5218 (*Huntsman Advance Materials*, USA) was dissolved in a certain volume of dicloromethane (CH_2Cl_2). The MOF solutions were also prepared in separate vials. The prepared solutions were sonicated for 4 hours and agitated on magnetic stirrers for 24 hours. Both solutions were then mixed together and agitated for 1 hour. Finally, the solutions were poured into Teflon plates and left in desiccators for a slow evaporation of the solvent.

2.2.3 Membrane characterisation

2.2.3.1 Contact angle

Contact angles measurements were carried out by the sensible drop method using a goniometer, as described by Couto et al., (2013), in order to evaluate the hydrophilic character of the membranes prepared. A drop of water was deposited on the membrane's surface using a syringe. Images of the drop were acquired and processed by the software Cam2008 (KSV instruments, Finland) calculating the angles both on the right and left side of the drop. Each measurement contemplates a total of 10 frames with 1 second interval between them. Multiple replicates were performed for each membrane.

2.2.4 Gas permeation experiments

2.2.4.1 Single gas permeability

The pure gas permeability of the mixed matrix membranes for CO_2 and CH_4 was assessed using the equipment described by Neves et al., (2010) and schematised in Figure 2.2. These gases were chosen for permeation studies since these are the most important in biogas upgrading. The apparatus is composed of a stainless cell with two identical compartments separated by the membrane to be tested. In order to measure the pure gas permeability, both compartments (feed and permeate) were initially pressurised with the gas. Then, the permeate outlet was open until a driving force of approximately 0.7 bar between the compartments was established. The pressure change in both compartments was recorded over time using two pressure indicators (Druck PCDR 910 models 99166 and 991675, UK) and the software LabVIEW (National Instruments, USA). Experiments were performed at a constant temperature of 30 ºC, maintained by a thermostatic water bath (Julabo, Model EH, Germany).

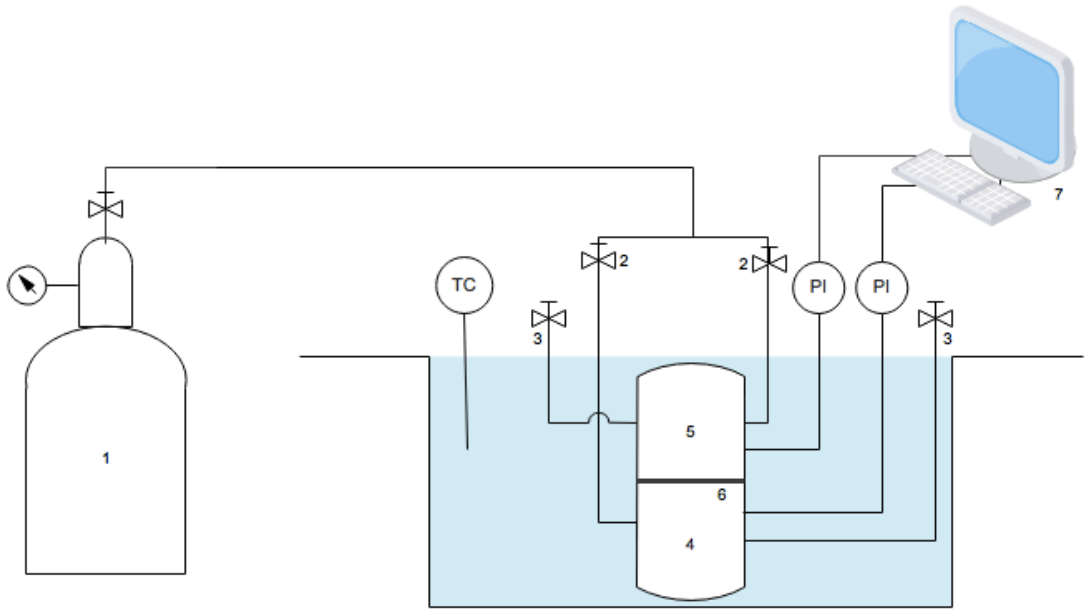

Figure 2.2 Schematic representation of the single gas permeation instalation. (1) Test gas; (2) Inlet valves; (3) Outlet valves; (4) Feed; (5) Permeate; (6) Membrane; (TC) Temperature contol; (PI) Pressure indicators

2.2.5 Calculations

Permeability is calculated according to Equation 8, where Δp_0 and Δp correspond to the difference between the pressure in the feed and permeate compartments (bar) at the initial instant (t_0) and over time, respectively, P is the membrane permeability ($m^2\ s^{-1}$) and l is the membrane thickness (m). β is the geometric parameter and is calculated following Equation 9, where A is the membrane area (m^2) and V_{feed} and V_{perm} correspond to the volumes of feed and permeate compartments (m^3), respectively. Permeability was obtained from the slope of the representation $1/\beta \ln(\Delta p_0/\Delta p)$ versus t/l.

$$\frac{1}{\beta} \ln\left(\frac{\Delta p_0}{\Delta p}\right) = P \frac{t}{l} \quad \text{(Equation 8)}$$

$$\beta = A \times \left(\frac{1}{V_{feed}} + \frac{1}{V_{perm}}\right), \text{ in } m^{-1} \quad \text{(Equation 9)}$$

The ratio of the permeabilities of two pure gases, in this case CO_2 and CH_4, corresponds to the ideal selectivity (α) as shown in Equation 10.

$$\alpha_{CO_2/CH_4} = \frac{P_{CO_2}}{P_{CH_4}} \quad \text{(Equation 10)}$$

3

Results and discussion

3.1 Fruit pulp waste characterisation

Peach, raspberry and white guava pulp wastes were used as substrate in this study. Substrate characterisation was essential for the adjustment of parameters such as influent dilution. The physiochemical composition of the fruit pulp wastes used in this work is presented in Table 3.1.

Table 3.1 Characterisation of the different fruit pulp wastes treated in the two-stage AD system.

Parameter	Peach	Raspberry	White guava
pH	4.98 ± 0.07	3.45 ± 0.03	4.18 ± 0.05
TSS (g L^{-1})	24.5 ± 1.0	220.3 ± 16.5	57.8 ± 3.3
VSS (g L^{-1})	22.9 ± 0.6	216.6 ± 16.9	55.6 ± 3.3
TS (g L^{-1})	44.2 ± 2.5	423.5 ± 33.6	72.5 ± 0.5
VS (g L^{-1})	39.5 ± 2.0	414.2 ± 43.8	67.3 ± 1.4
TS (%)	4.8 ± 0.2	36.0 ± 2.8	8.2 ± 0.2
VS (%)	4.2 ± 0.2	35.2 ± 2.6	7.6 ± 0.0
TCOD (g COD L^{-1})	107.7 ± 13.3	574.5 ± 41.1	100.8 ± 12.5
SCOD (g COD L^{-1})	75.8 ± 6.4	523.3 ± 15.9	63.0 ± 4.5
Total sugar (g COD L^{-1})	12.4 ± 2.2	383.4 ± 59.5	44.3 ± 7.1
Soluble sugar (g COD L^{-1})	1.6 ± 0.4	342.3 ± 82.8	39.6 ± 10.6
FP (g COD L^{-1})	78.7 ± 4.4	22.0 ± 3.3	6.5 ± 0.1
Total protein (g L^{-1})	7.5 ± 1.0	28.1 ± 1.2	9.0 ± 0.2
NH_4-N (mg N L^{-1})	440.5 ± 31.0	117.8 ± 15.1	55.7 ± 9.7
PO4-P (mg P L^{-1})	177.6 ± 10.9	250.7 ± 25.9	< 16.0

Although all substrates were fruit pulp wastes, their composition was different (Table 3.1). The amount of organic matter, in terms of COD, present in the raspberry pulp waste was approximately 5 times higher compared to the peach and white guava pulp wastes. Hence, considering that the influent concentration was maintained at a constant concentration of around 24 g COD L^{-1}, a higher dilution was needed for the raspberry pulp waste, so a longer time was needed to treat the same amount of waste. The portion of SCOD in each pulp was also different. The SCOD/TCOD ratios were of around 70% for peach, 91% for raspberry and 63% for white guava. As soluble organic components are readily available for acidogenic microorganisms (Bouallagui et al., 2005), higher SCOD/TCOD ratios may contribute positively for the process. The wastes presented high VS/TS ratios of around 91%, 98% and 93% for peach, raspberry and white guava pulp waste, respectively. These high VS/TS ratios indicate a high organic matter content, making these wastes and FW, in general, suitable substrates for AD (Zhao et al., 2016).

Nutrients concentrations (PO_4-P and NH_4-N) were substantially different between wastes. For all pulp wastes, nutrients were added to the influent in order to avoid nutrient limitation. Although raspberry pulp waste had higher protein concentration than the other two substrates, its total organic matter concentration was also higher, so the protein content was similar for all pulp wastes. The sugar and FP content, i.e. the sum of HOrgs and EtOH concentrations were different between wastes. Sugar content was 11.5 ± 2.1%, 66.7 ± 10.4% and 44.0 ± 7.0%, whereas the FP content was of 73.1 ± 4.1%, 3.8 ± 0.6% and 6.5 ± 1.0% for peach, raspberry and white guava pulp waste, respectively. These

differences could be related with the composition of the different fruits but also with the different pulp degradation stages. A higher degradation state was associated with high HOrgs content and low sugar content. Thus, as raspberry pulp waste was mainly composed by sugars it was considered to be in a low degradation state while peach pulp waste was mostly composed by HOrgs, suggesting a high degradation state.

3.2 Acidogenic fermentation – The effect of pH and HRT/OLR changes when treating different substrates

In the present study, different pH values (4.5, 5.0, 5.5) and HRTs (1 and 2 days) were tested when treating different fruit pulp wastes in the acidogenic reactor in order to study the effect of the operational conditions on the reactor's performance. OLR variations were associated with HRT changes since influent COD concentration was maintained at 24.5 ± 3.2 g COD L^{-1}.

Due to the partial re-inoculation on the 15th day of operation, an increase in CH_4 production was observed in the acidogenic reactor. The latter was due to the visible presence of methanogens as confirmed by FISH analysis (Figure 3.1 A). CH_4 content remained high during the start-up (pH of 5.5 and HRT of 4 days) and the second condition tested (pH of 5.5 and HRT of 2 days). The selection of fermentative bacteria in the first reactor is essential in two-stage anaerobic systems (Gonzalez-Martinez et al., 2016) thus, the pH was further decreased to 4.5. After twelve days, the CH_4 content in the produced gas decreased from 80.1% to 27.4%. The reduction of methanogenic population was also confirmed by FISH analysis (Table 3.4 and Figure 3.1). This reduction might also be associated with the longer time of operation and not uniquely with the change of conditions.

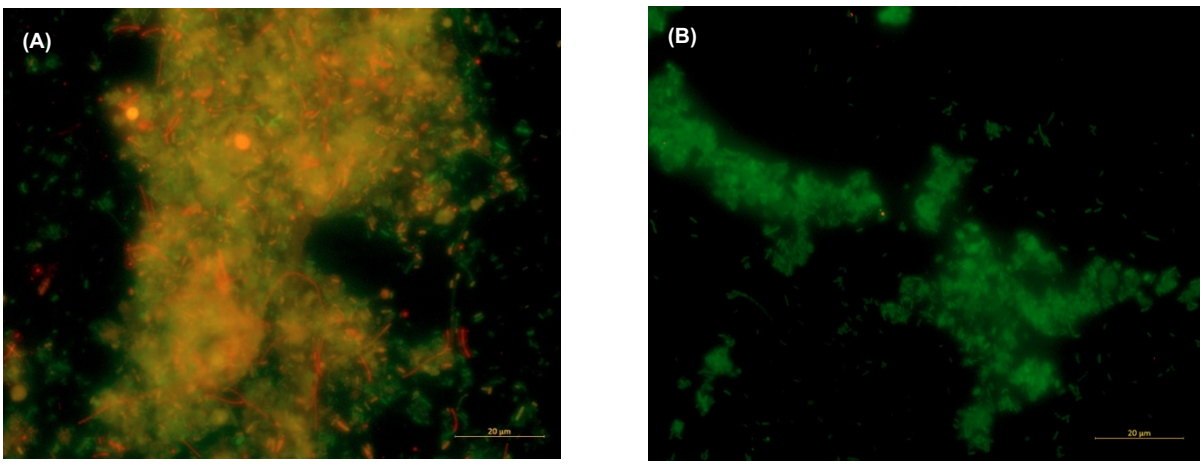

Figure 3.1 FISH image of the abundance of Archaea targeted by ARC915 on (A) day 16 (the day after the partial re-inoculation using peach pulp waste at pH 5.0 and HRT of 4 d) and on (B) day 42 (middle of the condition using peach pulp waste at pH 4.5 and HRT of 2 d). Archaea are shown in red and bacteria in green. Bar = 20 µm.

It is important to point out that after thawing and preparation of the influent, the latter (especially for raspberry) suffered a continuous degradation by feed-associated microorganisms. This degradation was observable by a reduction of sugar content and an increase of FP concentrations (mainly HLac and EtOH) present in the influent. Hence, the acidogenic reactor's influent composition was not stable throughout the reactor operation as shown in Figure 3.2. The instability in the influent composition also occurs in an industrial setting, thus the present lab-scale work mimics a real full-scale situation. The characterisation of each fruit pulp waste influent is presented in Table 3.2.

Table 3.2 Acidogenic influent characterisation for each fruit pulp waste.

Parameter	Peach	Raspberry	White guava
TSS (g L^{-1})	6.1 ± 1.6	2.8 ± 1.1	8.7 ± 0.5
VSS (g L^{-1})	5.0 ± 2.3	2.7 ± 1.1	8.1 ± 0.8
TCOD (g COD L^{-1})	25.4 ± 3.3	24.1 ± 3.4	22.5 ± 2.2
SCOD (g COD L^{-1})	16.9 ± 1.6	21.1 ± 2.5	13.7 ± 1.3
Total sugar (g COD L^{-1})	3.3 ± 1.2	7.5 ± 4.7	4.6 ± 1.3
FP (g COD L^{-1})	17.3 ± 2.5	12.6 ± 6.6	12.0 ± 2.6
Total protein (g L^{-1})	2.0 ± 0.6	1.2 ± 0.3	1.9 ± 0.4
NH_4-N (mg N L^{-1})	184.7 ± 39.0	134.2 ± 70.2	168.8 ± 46.7
PO_4-P (mg P L^{-1})	59.2 ± 11.7	34.6 ± 14.7	38.6 ± 13.5

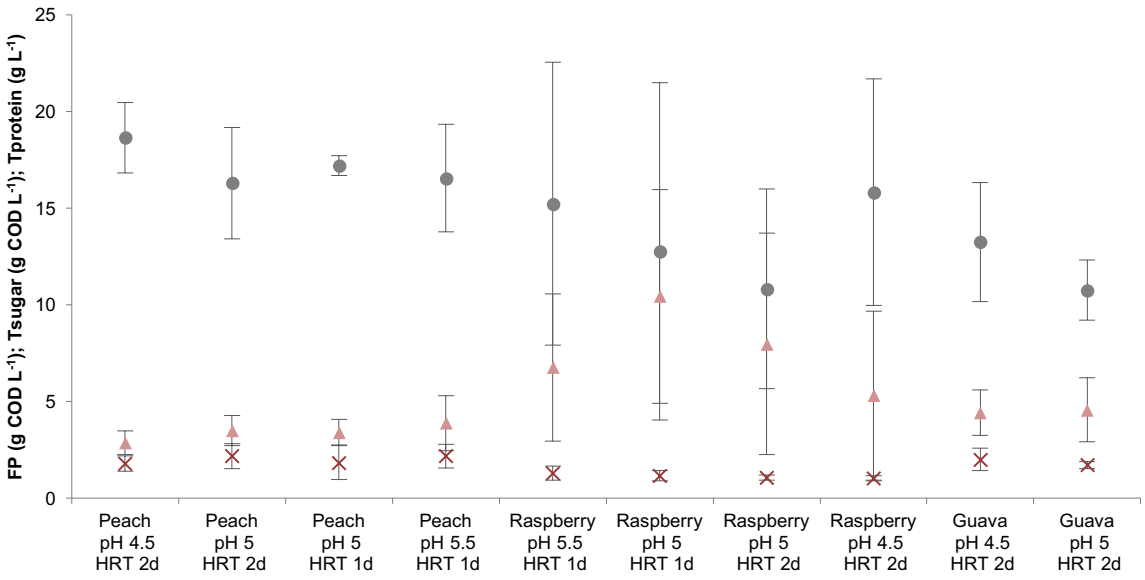

Figure 3.2 Average FP (●), total sugar (Tsugar) (▲) and total protein (Tprotein) (×) concentrations in the influent in every condition tested in the acidogenic reactor (conditions presented in chronological order). Error bars represent one standard deviation.

After biomass acclimatisation, four conditions were tested for the treatment of peach and raspberry pulp wastes: (1) pH 4.5 and HRT of 2 days; (2) pH 5 and HRT of 2 days; (3) pH 5 and HRT of 1 day and (4) pH 5.5 and HRT of 1 day. White guava pulp waste treatment was tested under conditions (1) and (2). Results regarding the acidification degree, sugar and protein removal and gas production are summarised in Table 3.3.

Table 3.3 Acidogenic reactor's performance in different operational conditions after biomass acclimatisation (conditions are not shown in chronological order).

Substrate	Conditions			Acidification degree (%)	Sugar removal (%)	Protein removal (%)	Gas production (L d^{-1})
	pH	HRT (d)	OLR (g COD L^{-1} d^{-1})				
Peach	4.5	2	11.9 ± 1.1	68.1 ± 11.3	93.8 ± 1.0	73.9 ± 10.5	1.2 ± 0.8
Raspberry			11.6 ± 0.5	74.6 ± 10.6	95.2 ± 3.7	67.0 ± 4.6	8.5 ± 4.1
White guava			11.9 ± 0.9	57.1 ± 6.7	97.5 ± 1.0	78.1 ± 8.5	4.5 ± 2.4
Peach	5.0	2	12.9 ± 2.4	53.7 ± 11.3	96.9 ± 1.9	78.0 ± 4.7	1.1 ± 0.7
Raspberry			11.9 ± 0.8	75.9 ± 8.6	97.8 ± 1.7	68.5 ± 5.6	8.5 ± 2.9
White guava			10.7 ± 1.0	58.0 ± 6.4	97.6 ± 0.7	76.5 ± 3.1	3.8 ± 1.7
Peach	5.0	1	25.7 ± 0.9	61.8 ± 6.8	97.6 ± 0.9	75.3 ± 9.8	2.0 ± 1.7
Raspberry			23.7 ± 3.1	76.4 ± 9.6	97.3 ± 2.9	64.8 ± 11.0	13.2 ± 5.9
Peach	5.5	1	24.1± 1.3	62.0 ± 11.4	97.6 ± 1.8	80.4 ± 3.6	1.3 ± 0.7
Raspberry			24.5 ± 4.2	72.5 ± 11.2	96.4 ± 2.4	66.3 ± 11.1	8.4 ± 4.1

3.2.1 Sugar and protein removal

For all conditions, an efficient sugar removal was obtained (93.8 – 97.8%) (Table 3.3), which resulted in residual sugar concentrations between 0.03 – 0.26 g L^{-1}. This suggests a stable operation throughout every condition tested. Thus, substrate shifts and HRT/OLR changes in the range studied did not affect the sugar removal. The range of pH values studied is considered optimal for acidogenic bacteria (Voelklein et al., 2016 and references therein) and variations within this range did not seem to affect sugar removal. Chu et al., (2008) observed similar carbohydrates' removal efficiencies (92%) in the acidogenic stage while treating food waste (carbohydrate content of around 46%) at pH 5.5, higher OLR (64.4 g COD L^{-1}) and similar HRT (1.3 d). Raspberry influent had the highest sugar concentration and variation among the three substrates (Figure 3.2) but its fermentation resulted in similar final sugar concentrations when compared to the other two substrates tested. This shows that the microorganisms were able to consume almost all sugar in a wide range of initial concentrations (1.1 – 19.8 g L^{-1}).

Protein removal was similar in every condition tested for each substrate fermentation and overall protein removal varied between 64.8 ± 11.0% – 80.4 ± 3.6% (Table 3.3). Protein concentration inside the reactor varied between 0.25 – 0.72 g L^{-1} throughout the operation. Protein removal was lower than sugar removal regardless of the condition tested. This can be related with the fact that protein degradation is slower than sugar degradation (Kobayashi et al., 2012 and references therein). Yang et al., (2015) specifically studied the degradation of proteins and carbohydrates during sludge anaerobic digestion in batch tests and concluded that not only the carbohydrate degradation was faster and more efficient, it occurred prior to protein degradation, creating a lag-phase of 3 days for protein degradation. Moreover, it was suggested that the glucose produced during the fast carbohydrate degradation had repressed protease formation. Hence, when operating at such short HRTs (1 – 2 days), protein removal was not maximised. Nonetheless, most of the protein not removed in the acidogenic phase was later removed in the methanogenic phase.

As mentioned in the Materials and methods section, the influent was supplemented with a nutrient solution to avoid nutrient limitation for the microorganisms in the first stage as well as for the

microorganisms in the methanogenic reactor, since the nutrients will be provided solely by the acidogenic fermentation broth. PO_4-P and NH_4-N concentrations were measured in order to monitor microbial growth/decay and results are shown in Figure 3.3. There was no indication of nutrient limitation throughout the operation except for a small period of time after the first substrate shift (from day 111 to 127) as it will be discussed in section 3.2.4. A lower concentration of nutrients in the fermentation broth comparing to the influent is associated with nutrient uptake and microorganisms' growth. On the other hand, if the concentration in the fermentation broth is higher than the concentration in the influent, this may suggest cell death since these nutrients are released during cell lysis. However, the release might not be associated with cell death. For instance, ammonia production occurs during protein degradation which may explain an increase in NH_4-N concentration (Jiang et al., 2013; Wang et al., 2014).

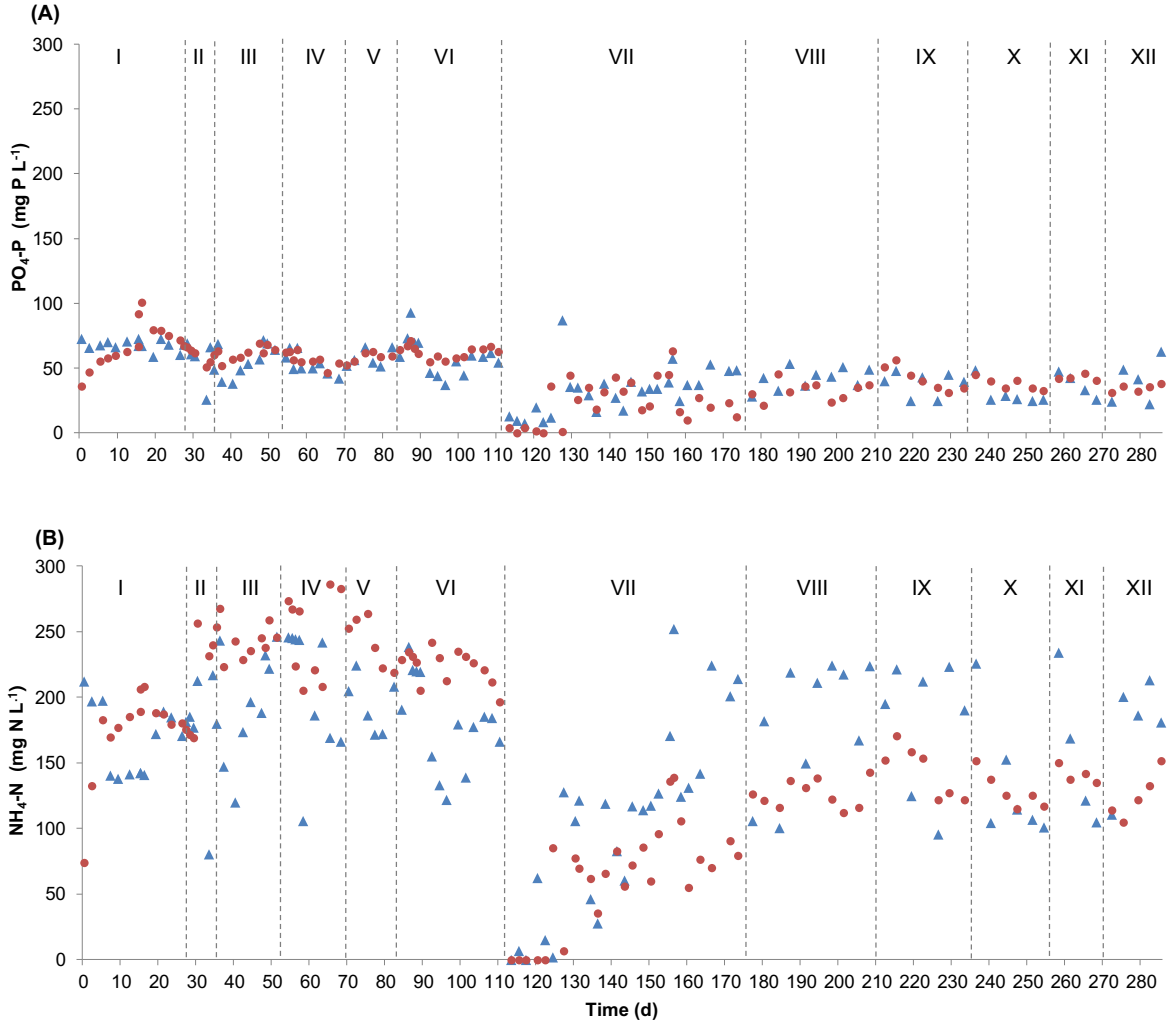

Figure 3.3 (A) Phosphorus and (B) ammonium concentrations in the influent (▲) and in the fermentation broth (●) during the 285 days of operation under different operational contidions: (I) Peach, HRT 4 d, OLR 7.0 ± 0.9 g COD L^{-1} d^{-1}; pH 5.5; (II) Peach, HRT 2 d, OLR 11.9 ± 1.5 g COD L^{-1} d^{-1}; pH 5.5; (III) Peach, HRT 2 d, OLR 11.9 ± 1.1 g COD L^{-1} d^{-1}; pH 4.5; (IV) Peach, HRT 2 d, OLR 12.9 ± 2.4 g COD L^{-1} d^{-1}; pH 5; (V) Peach, HRT 1 d, OLR 25.7 ± 0.9 g COD L^{-1} d^{-1}; pH 5; (VI) Peach, HRT 1d, OLR 24.1 ± 1.3 g COD L^{-1} d^{-1}; pH 5.5; (VII) Raspberry, HRT 1 d, OLR 24.5 ± 4.2 g COD L^{-1} d^{-1}; pH 5.5; (VIII) Raspberry, HRT 1 d, OLR 23.7 ± 3.1 g COD L^{-1} d^{-1}; pH 5; (IX) Raspberry, HRT 2 d, OLR 11.9 ± 0.8 g COD L^{-1} d^{-1}; pH 5; (X) Raspberry, HRT 2 d, OLR 11.6 ± 0.5 g COD L^{-1} d^{-1}; pH 4.5; (XI) White guava, HRT 2 d, OLR 11.9 ± 0.9 g COD L^{-1} d^{-1}; pH 4.5; (XII) White guava, HRT 2d, OLR 10.7 ± 1.0 g COD L^{-1} d^{-1}; pH 5.

3.2.2 FP concentration and profiles

Raspberry pulp waste fermentation seemed to have resulted in slightly higher FP concentrations comparing peach and white guava pulp waste fermentation when operating at the same pH and HRT/OLR (Figure 3.4). However, one exception was verified at pH 4.5 and HRT of 2 days where FP concentrations were similar for raspberry and peach pulp waste fermentation. Since the influent COD concentration was maintained constant throughout the study (24.5 $\pm$ 3.2 g COD L^{-1}), it should not be the cause for the variation of FP concentrations in the fermentation broth. Moreover, the SCOD portion of the total influent COD was different between substrates as referred above in section 3.1 which can indicate a different biodegradability between the wastes. Raspberry pulp waste has the highest portion of SCOD (91%) followed by peach (70%) and white guava pulp wastes (63%) which is coherent with the results of FP concentration in the fermentation broth. Acidification degrees obtained for raspberry pulp waste fermentation also seemed to be slightly higher compared to peach and white guava pulp waste fermentations in the same operational conditions (Table 3.3 and Figure 3.4). Overall acidification degree varied between 53.7% and 76.4% when all FP are considered and between 17.3% and 38.7% when only VFAs are considered. Voelklein et al., (2016) obtained acidification degrees between 64.1% and 88.5% (considering all FP) treating food waste at OLRs between 8.8 – 21.9 g COD L^{-1} d^{-1}. However, those acidification degrees were calculated based on the soluble COD whereas in this work they were calculated based on the total COD, which may explain the lower acidification degree obtained in the present work. Bouallagui et al., (2004) obtained constant acidification degrees (38.9% – 44.4%) when treating FVW at OLRs between 3.7 – 10 g COD L^{-1} d^{-1} and considering only VFAs. The fact that EtOH is one of the main FP obtained in the present study might contribute for lower acidification degrees when only VFAs are considered (Equations 2 and 3 in section 2.1.5).

FP concentration in the fermentation broth was maintained approximately constant after HRT/OLR changes (Figure 3.4) which indicates that the smaller HRT (1 day) was sufficient to ensure an efficient acidification. Furthermore, there seemed to be no association between FP concentration and the different HRT/OLR tested in the present work. Paudel et al., (2017) also did not observe significant differences in VFA production related to HRT/OLR changes during the co-digestion of food waste and brown water. The authors accounted hydrolysis/solubilisation as the main parameter affecting VFA conversion, which supports the hypothesis that the substrate composition may have higher influence on VFA production than HRT/OLR changes. Conflicting results regarding this topic have arisen in the literature. While in some studies HRT affected VFA production and profile, other studies did not show this association. It has been suggested that VFA production during the fermentation of simpler and soluble substrates is usually less affected by HRT than the fermentation of more complex and recalcitrant substrates (Demirel and Yenigun, 2004 and references therein; Fang and Yu, 2001 and references therein).

In the present study, pH also did not seem to affect FP concentration (Figure 3.4). Zheng et al., (2015) studied the effect of pH on the fermentation of FVW and observed no difference in FP concentration at pH 4 and 5. However, a lower FP concentration was observed at pH 6. The authors hypothesised that

methanogens can survive at pH 6 therefore one part of the VFAs would be converted to CH_4 or CO_2. The production of these gases, especially CO_2, may explain the fact that the SCOD in the fermentation broth in the present study was constantly lower than the influent's COD concentration. SCOD accounts for FP, sugar, protein and other products concentrations. Besides gas production, other factors which may contribute for the difference between COD concentrations are (1) non-hydrolysable substrate, (2) substrate used for biomass growth, (3) errors introduced by the analysis. However, there is not sufficient data to further explore these possibilities.

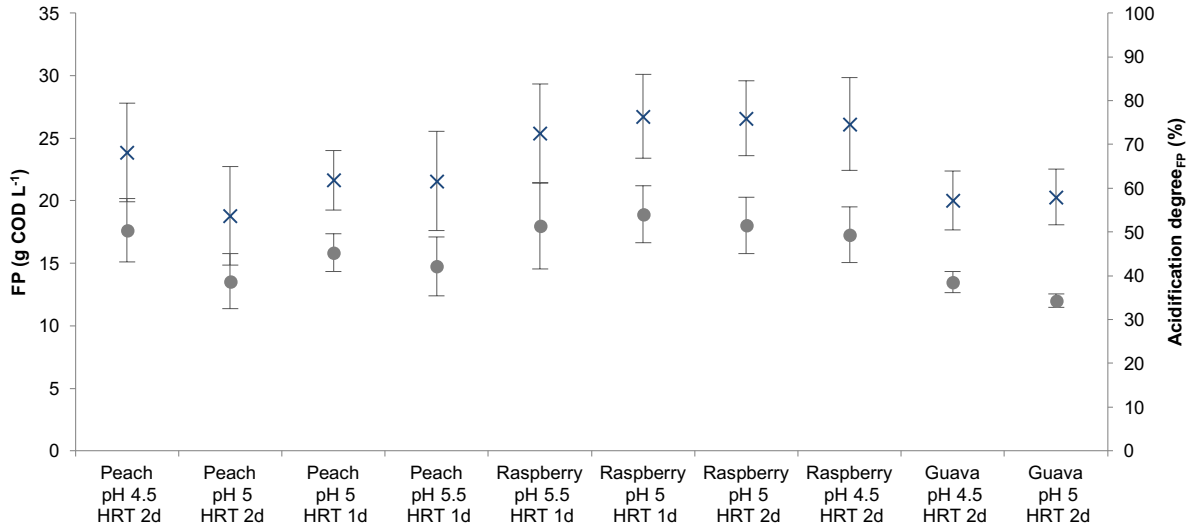

Figure 3.4 Average FP concentration in the fermentation broth (●) and acidification degree (×) in every condition tested in the acidogenic reactor (conditions presented in chronological order). Error bars represent one standard deviation.

Besides FP concentration, FP profiles were also analysed in order to find some possible associations with the operational conditions imposed and to assess the reactor stability. The influent and fermentation broth FP profiles in every condition tested after biomass acclimatisation are represented in Figure 3.5. The HOrgs analysed were HLac, HAc, HPr, HBut and HVal. These corresponded to the HOrgs present in higher amounts, however other compounds could be present but in low/residual concentrations. The FP profiles of each fruit pulp waste influent remained fairly stable throughout the conditions tested, as shown in Figure 3.5 A, even though the total FP concentration the influent varied (especially in raspberry), as referred above and shown in Figure 3.2. The main FP present in every influent was EtOH. HLac was also detected in all influents possibly due to the presence of the lactic acid bacteria, which is indigenous of food waste (Kim et al., 2009; Paudel et al., 2017). HAc and HPr concentration was only significant in the peach pulp waste probably due to the high degradation state of this substrate, as referred above in section 3.1.

EtOH corresponded to the FP in higher abundance in the fermentation broth, followed by HBut, HAc and HVal in every condition tested. EtOH was already the main component in the influent so it is difficult to conclude whether and when it was produced, consumed and remained non-metabolised. Apart from HLac, FP profiles were similar between the different conditions tested (Figure 3.5 B).

Furthermore, FP profiles in the fermentation broth remained mostly stable within each condition. Still, some fluctuations were observed especially during raspberry pulp waste fermentation which seemed to be associated with the influent composition variation or to some stress conditions. For instance, HLac concentration peaks were coincident with different stress situations that happened during the reactor's operation, such as a sudden pH (day 198) or temperature (day 240) decrease or the re-start of feeding after electricity issues (day 148). After that, HLac concentration returned to the values obtained before. Apart from these situations, HLac concentration was minimal or close to zero in most conditions tested (0 – 3.8% among the FP measured), except for one condition: raspberry pulp waste fermentation at pH 5 and HRT of 1 day (≈14%). HLac has the potential to be further converted into HPr in the methanogenic phase, so its production in the acidogenic phase is undesirable (Ren et al., 1997). HPr production should be reduced or avoided because its conversion to HAc and H_2 is the least thermodynamically favourable reaction (higher ΔG) and the most sensitive to H_2 partial pressure (Khanal, 2008; Zheng et al., 2015). In the present study, HPr concentration was always lower than HBut, HAc, HVal and EtOH. It is interesting to note that HPr production is the more energetically favourable for acidogens (Azbar et al., 2001) and nonetheless HPr is often found in lower concentrations in stable operating acidogenic reactors comparing to other FP (Bouallagui et al., 2004; Chen et al., 2015; Chu et al., 2008; Voelklein et al., 2016). Azbar et al., (2001) hypothesised that reduced pH associated with increased VFA concentration may stress the acidogens leading to the formation of products less favourable to them but more favourable for the latter CH_4 production. The authors state that, in phased systems, fermentation of complex substrates is usually directed to the production of HBut and EtOH comparing to HPr which is in accordance to the present study.

In this study, FP profiles were similar for all pH tested (4.5, 5.0, 5.5). Hence, no apparent correlation between pH and FP composition was observed. Some studies suggest that pH influences FP composition by promoting either metabolic pathway changes in the same microbial population or a shift in the dominant microbial population (Horiuchi et al., 2002; Wu et al., 2017). Three or four fermentation types are suggested in literature according to the FP profiles, most times associated with specific pH values. The designation of the fermentation type is usually associated with the dominant FP produced. In EtOH-type fermentation, EtOH and HAc are dominant and has been observed at pH 4.0 – 4.5. Mixed acid-type fermentation is considered when HAc is the main product and the remaining products have similar ratios (pH 4.5 – 5.0). HPr-type occurs if HPr percentage is higher than 15 – 20%, normally at pH 5.0 – 5.5. Finally, HBut-type fermentation happens at pH 5.5 – 6.5 with the predominance of HBut and HAc (Chen et al., 2015 and references therein; Zheng et al., 2015). However, this classification has not always shown to be confirmed. For instance, treating food waste at pH 5.5 resulted in a higher proportion of EtOH, HBut and HAc, i.e., a mixed type EtOH–HBut acid fermentation (Voelklein et al., 2016). On the other hand, Wu et al., (2017) observed a HBut-type fermentation of FVW at pH 5.0, 5.5 and 6.0 with very similar FP profiles. Still, at pH 4, the FP profile was considerably different showing an EtOH-type fermentation. Taking into account these examples, the relationship between pH and FP composition does not seem straightforward.

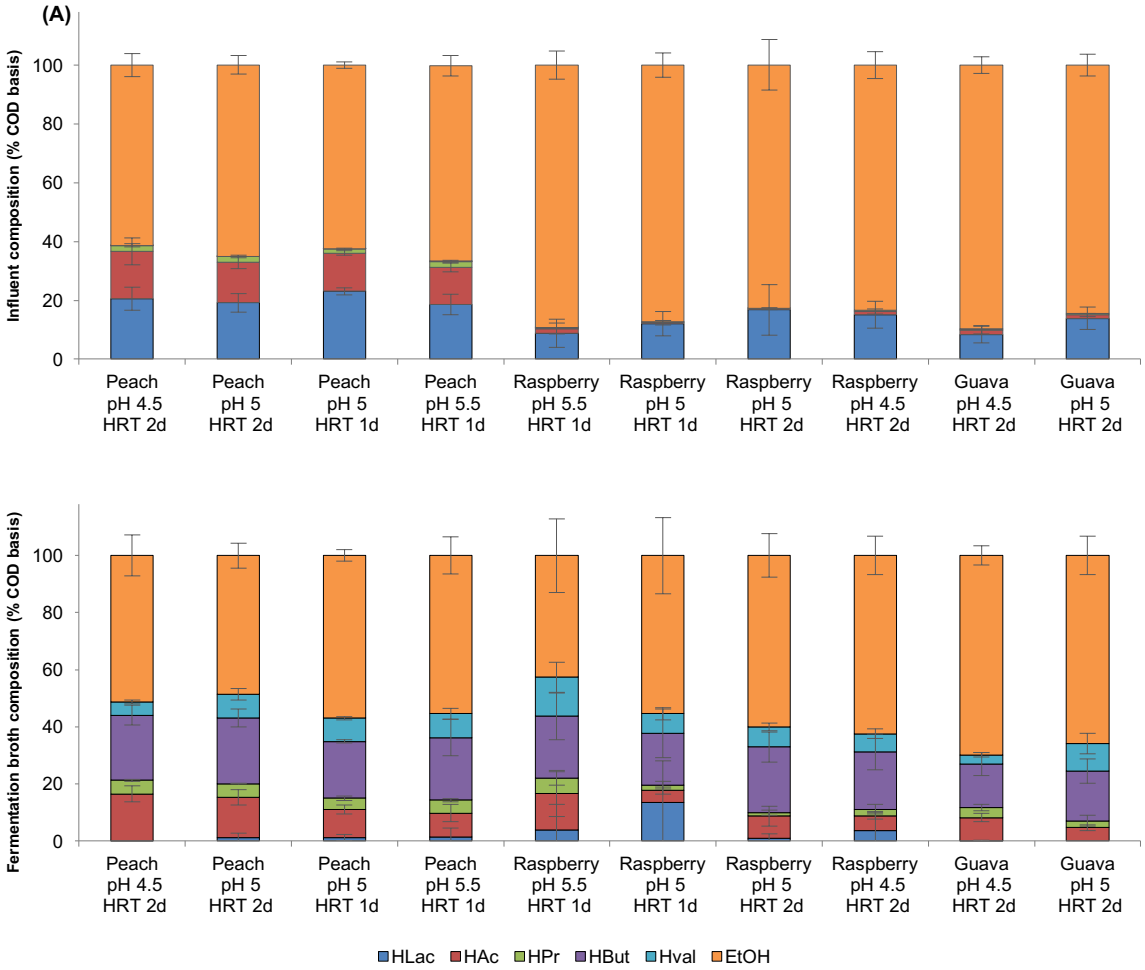

Figure 3.5 Average influent (A) and average fermentation broth (B) composition in terms of FP profiles obtained for each condition tested in the acidogenic reactor (conditions presented in chronological order). Error bars represent one standard deviation.

Considering these results, it can be concluded that it was possible to maintain a stable operation throughout the conditions tested. FP concentration and, consequently, acidification degree seemed to be more affected by the substrate characteristics rather than by HRT/OLR and pH changes. The main FP present in most conditions were EtOH, HBut and HAc, which presents an advantage for methanogenic operation. HLac production seemed to be associated with stress conditions, however further studies are needed to confirm it.

3.2.3 Gas production and composition

Raspberry pulp waste fermentation resulted in the highest rate of gas production in the acidogenic reactor whereas peach pulp waste was associated with the lowest gas production and intermediary production was obtained for white guava pulp waste (Figure 3.6). The maximal gas production of 13.2 ± 5.9 L gas d^{-1} was obtained when treating raspberry pulp waste at HRT of 1 day and pH 5. Although the overall gas production was different between the substrates, the variation associated with operational changes (pH, HRT/OLR) was similar in all of them. For peach and raspberry pulp wastes, it was possible to evaluate the effect of changing the HRT (and consequently, the OLR) from 2 days to 1 day at pH 5. There was an average increase in gas production by 1.8 times for peach and by 1.6 times for raspberry. Paudel et al., (2017) studied the effect OLR changes, between 17.7 – 106 g VS L^{-1} d^{-1}, in gas production in a two-stage anaerobic co-digestion system treating food waste and brown water at similar HRTs and pH to this study. The authors also observed a significant increase in gas production in the acidogenic reactor when HRT decreased (and OLR increased).

Gas production was similar at pH 4.5 and 5 at a HRT of 2 days for each of the substrates tested (Figure 3.6). This suggests that, similarly to FP concentration profile, substrate composition has a stronger influence on gas production than the change of pH from 4.5 to 5. At a HRT of 1 day, gas production decreased by 1.5 times for both peach and raspberry pulp waste when operating at pH 5.5 in comparison to pH 5. Hence, it appears that at pH of 5.5, there was an alteration in the metabolic pathways which resulted in a lower gas production. Interestingly, gas production values at pH 4.5 and 5 at a HRT of 2 days were similar to the values obtained at pH 5.5 and HRT of 1 day when treating peach and raspberry pulp wastes. This suggests that smaller HRTs/higher OLRs do not necessarily translate to higher gas production at any pH. Rather, it seems that changing the pH from 4.5 – 5.0 to 5.5 might have an influence on gas production. However, to further support these conclusions, it would be necessary to test two other operational conditions: (1) pH of 4.5 and HRT of 1 day and (2) pH of 5.5 and HRT of 2 days in order to have information about every combination of pH and HRT/OLR of the range studied for each substrate. For white guava pulp waste, it would also be necessary to test the conditions which were only tested for peach and raspberry. Nevertheless, it is expected that the results follow the same trend as the ones concerning the other two substrates.

It was also possible to observe, especially when treating raspberry and white guava pulp wastes, that there was a decline in biogas production concomitant with the degradation of the influent as referred in section 3.2. While the influent fermented at 4ºC, the reactor was fed with a subsequent lower sugar concentration leading to subsequent lower gas production. A sharp increase in gas production was detected only a few minutes after the influent was changed to a fresh one. This resulted in a large gas production variation within each condition as shown by the standard deviation in Figure 3.6. This was not so noticeable when treating peach pulp as this waste was already in an advanced stage of degradation and its sugar content was already low. Sugar concentration seems to be directly impacting gas production in the acidogenic phase.

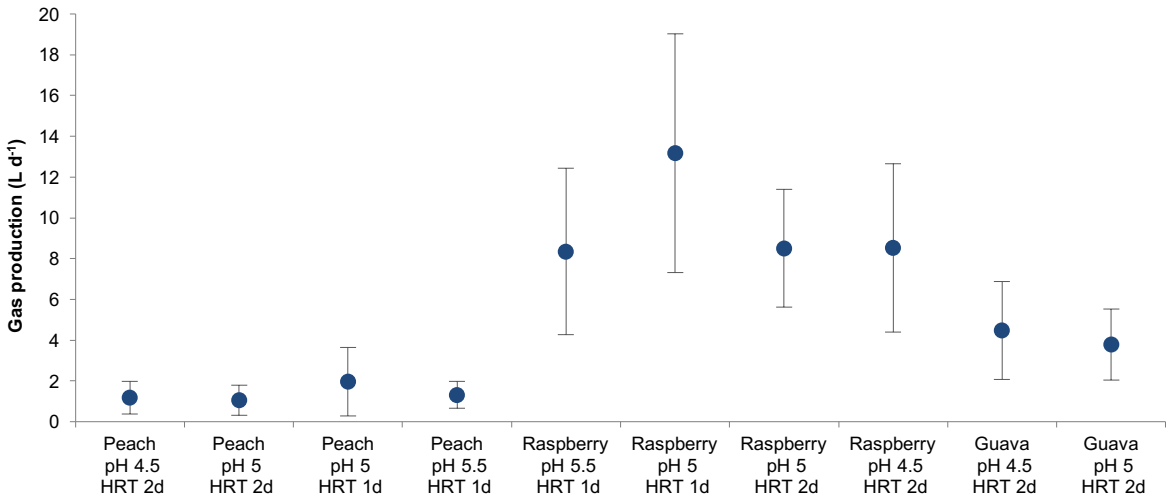

Figure 3.6 Average acidogenic gas production in each condition tested, in chronological order. Error bars represent one standard deviation.

The study of the acidogenic gas composition was not one of the initial main goals in this work, so GC analysis was only performed once a week in order to control the presence/variation in CH_4, CO_2 and H_2 content. As the acidogenic reactor was operated at low HRTs (1 – 2 days), some conditions were maintained for less than three weeks. Therefore, it was not possible to obtain three or more samples for each condition. Nevertheless, it was possible to raise some hypothesis which might be interesting to investigate in the future. The average gas composition in each condition is present in Figure 3.7. The main gas produced in every condition tested after biomass acclimatisation was CO_2 (52 – 89%), which is in agreement with previous studies (Bouallagui et al., 2004; Lindner et al., 2016; Shen et al., 2013). During peach pulp waste fermentation, H_2 was not detected while there was some CH_4 production (20 – 24%). Nevertheless, the non-detection of H_2 during peach pulp waste fermentation does not necessarily mean it was not produced. H_2 might have been consumed at a higher rate by hydrogenotrophic methanogens or homoacetogens for CH_4 or HAc production. Some studies have recognised that hydrogenotrophic methanogens are more tolerant to lower pHs and HRTs than acetoclastic methanogens (Kim et al., 2004; Solera et al., 2002). Therefore, these conditions might not have been enough to inhibit H_2 consuming methanogens. Still, the lower H_2 production could also be a consequence of the relatively slow biochemical pathways to produce H_2 from HLac (abundant in the influent) and HBut (abundant inside the reactor), as suggested by Kapdan and Kargi, (2006). On the other hand, during raspberry and white guava pulp waste fermentation, H_2 was detected while there was only a small or almost no production of CH_4. The higher sugar content on these two substrates comparing to peach pulp places them as more suitable substrates for H_2 production (Kim et al., 2011). Maximum H_2 content was detected during raspberry pulp waste fermentation at pH 5 and HRT of 1 day, which was the same condition for maximal gas production. The lowest CH_4 production during raspberry feeding onwards was detected after the pH was changed to 4.5. This again shows the susceptibility of methanogenic archaea to low pH values even though some methanogenic activity was present in the previous conditions tested.

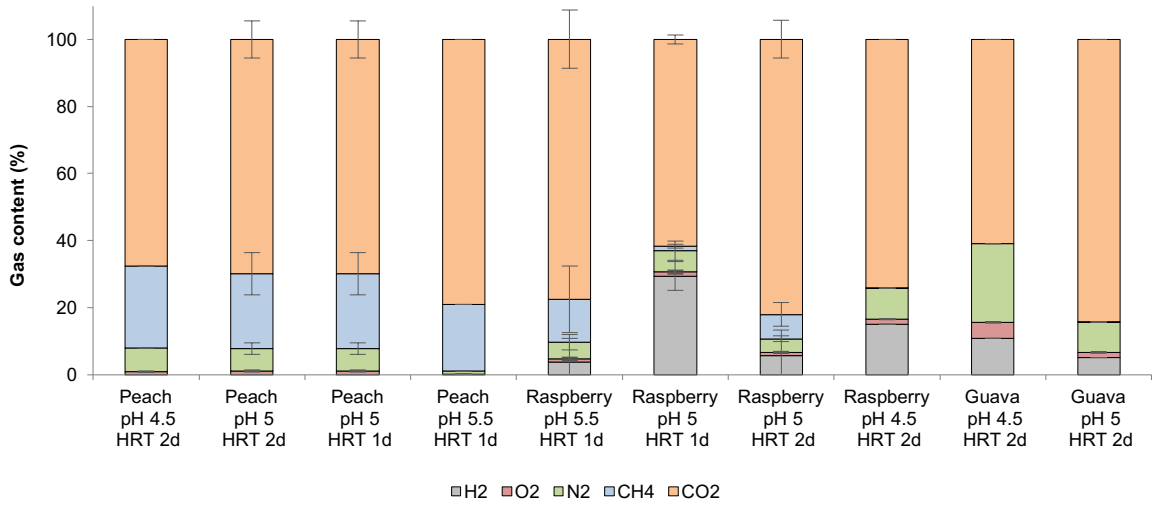

Figure 3.7 Average acidogenic gas composition in each condition tested, in chronological order. Error bars represent one standard deviation. The absence of error bars indicates that the average presented was calculated based on < 3 samples. The N_2 and O_2 content in the condition treating white guava ah pH 4.5 and HRT of 2d may be overvalued since there was only one sample which was taken after a purge of the settler.

FISH analysis was performed in order to assess the stability of the microbial community throughout the operation. The qualitative results showing the relative abundance of the groups of microorganisms studied are presented in Table 3.4. The microbial community of the acidogenic reactor was fairly stable throughout the operation, with exception of two situations. The first is the change in the archaea's relative abundance during biomass acclimatisation (mentioned at the beginning of section 3.2) and the second is the significant increase in abundance of Firmicutes from day 136 onwards comparing to the earlier time of operation. *Clostridium* species, which belong to the Firmicutes filo, are the main anaerobic H_2 producers (Fang et al., 2002; Kapdan and Kargi, 2006; Kim et al., 2011). The increase in Firmicutes relative abundance is concomitant with the condition where H_2 production was detected for the first time. Firmicutes remained present or abundant throughout the conditions where H_2 production was detected. Although it was not possible to confirm that this increase was related to *Clostridium* species, these results seem to be in agreement with the gas analysis. In order to confirm the effective increase in H_2 producing bacteria and its identification, it would be necessary to perform, for instance, phylogenetic studies based on 16S rDNA. The onset of H_2 production was probably a consequence of the adaptation to a substrate richer in sugar (i.e. raspberry), since carbohydrates are the preferred substrates for H_2 production (Rafieenia et al., 2017 and references therein). Indeed, it has been observed that the potential for H_2 production of a certain organic waste is greatly dependent on its carbohydrate content (Kim et al., 2011).

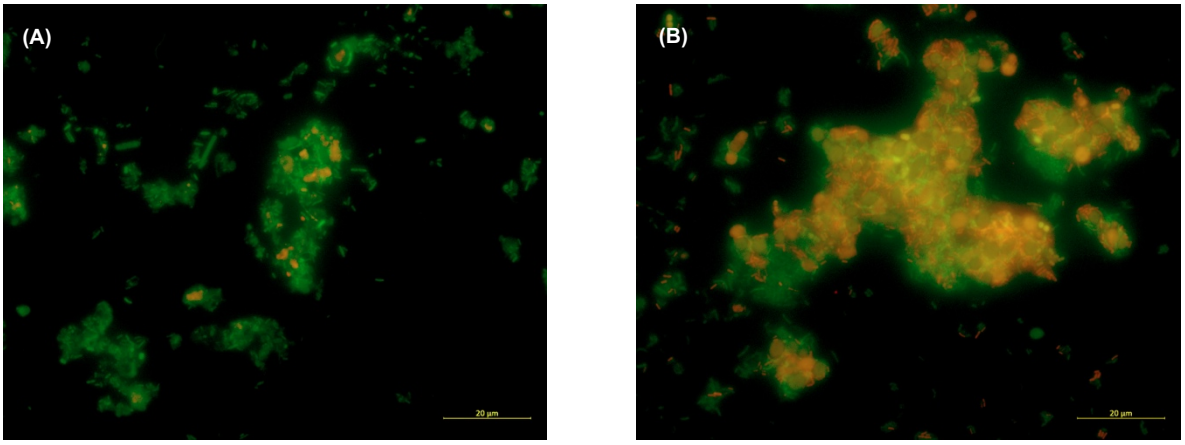

Figure 3.8 FISH image of the abundance of Firmicutes targeted by LGC0355 on (A) day 108 (the end of the condition treating peach pulp waste at pH 5.5 and HRT of 1 d) and on (B) day 136 (the middle of the condition treating raspberry pulp waste at pH 5.5 and HRT of 1 d). Firmicutes are shown in red and other bacteria in green. Bar = 20 μm.

Table 3.4 Microbial community in the acidogenic reactor – qualitative analysis based on FISH.

		Conditions		Probes (microorganisms)							
Day	**Substrate**	**pH**	**HRT**	ALF969 (Alphaproteiobacteria)	BET2a (Betaproteobacteria)	GAM2a (Gammaproteobacteria)	DELTA495 (Deltaproteobacteria)	LGC0355 (Firmicutes)	BAC303 (Bacteroidaceae, Prevotellaceae)	CF39a (Cytophaga, Flavobacteria)	ARC915 (Archaea)
1	First inoculation	5.5	4	+	+ –	+ +	+ –	+ –	+	+ –	+
16	Partial re-inoculation	5.5	4	+ –	+	+ –	+ –	+ –	+ –	+	+ +
26 (end)	Peach	5.5	4	+ –	+ –	+ –	+ –	+ –	+ –	+ +	+
42 (middle)	Peach	4.5	2	+ –	–	+ –	+ –	+ –	–	+ –	+ –
63 (middle)	Peach	5.0	2	+ –	–	–	+ –	+ –	+ –	+ –	+ –
68 (end)	Peach	5.0	2	+ –	+ –	–	–	+ –	+ –	–	+ –
84 (end)	Peach	5.0	1	+ –	–	–	–	+ –	+ –	–	+ –
108 (end)	Peach	5.5	1	+ –	–	–	+ –	+	+ –	–	+ –
136 (middle)	Raspberry	5.5	1	+ –	–	–	+ –	+ + +	–	+	+ –
174 (end)	Raspberry	5.5	1	+	–	–	–	+ +	–	+ –	+ –
208 (end)	Raspberry	5.0	1	+ –	+	+ –	+ –	+	+ –	–	+
234 (end)	Raspberry	5.0	2	+ –	+ –	+ –	+ –	+ +	+ –	–	+
256 (end)	Raspberry	4.5	2	+ –	+ –	+ –	+	+ +	+ –	+ –	+
271 (end)	White guava	4.5	2	+ –	+ –	+ –	+	+	+	–	+ –
285 (end)	White guava	5.0	2	+ –	+ –	+ –	+	+ +	+	–	–

(–) Non or almost non-existant; (+ –) Identifiied (1 – 5%); (+) Present (5 – 20%); (++) Abundant (20 – 50%); (+++) Extremely abundant (> 50%)
(middle), (end) refers to each condition time frame

From these results, it seems that the overall gas production was affected by substrate composition, OLR/HRT and pH. Higher sugar content led to higher gas production. Higher OLR and lower HRT also resulted in increased gas production at pH 5. Finally, more gas was produced when pH was maintained at 4.5 – 5.0 than at 5.5. CO_2 was the main gas produced in every condition while H_2 and CH_4 production seemed to be only promoted in certain conditions. CH_4 production suffered significant decreases in the two conditions when the pH was changed to 4.5. H_2 production seemed to be promoted by an increase in sugar content in the influent but it was not possible to associate H_2 production with pH and HRT/OLR changes.

3.2.4 Substrate shifts – The effect on FP composition and reactor stability

In the present study, two substrate shifts were tested: (1) peach to raspberry at pH 5.5 and HRT of 1 day and (2) raspberry to white guava at pH 4.5 and HRT of 2 days. The reactor presented a stable performance before the substrate shift.

Regarding the first substrate shift (from peach to raspberry pulp waste), it was difficult to maintain a constant raspberry influent COD concentration from the point of the substrate shift until the 127th day of operation (Figure 3.9 A). Thus, a variation in FP concentrations in the fermentation broth was observed during the same period (Figure 3.9 B). In spite of this, FP profiles were constant during those days. From day 129, it was possible to maintain a stable OLR and FP concentration in the fermentation broth. Therefore, the fluctuations on the FP concentration in the fermentation broth might have been caused by the influent variation and not necessarily an indication of reactor instability. In fact, other parameters, such as sugar removal and gas production suggest a fast adaptation of the system. After the FP concentration in the fermentation broth stabilised, the main difference between FP profiles of raspberry and peach pulp waste fermentation is an increase in HAc concentration in raspberry pulp waste fermentation. One important factor affected by the change from peach to raspberry influent concerns the nutrients concentrations. Immediately after the substrate change, the COD:N:P ratio was maintained at 100:0.5:0.1. However, as N and P concentration were close to zero until the 127th day (Figure 3.3), the ratio was changed to 100:1:0.2 in order to avoid nutrient limitation.

There seemed to be a slight decrease in FP concentrations in the fermentation broth after the second substrate shift (from raspberry to white guava pulp waste) as shown in Figure 3.10 B. However, the concentrations and profiles are constant immediately after the shift, which suggests a fast adaptation and bioreactor stability. Furthermore, high sugar removal was also maintained. The decrease in FP concentration may be associated with a lower SCOD/TCOD ratio and substrate biodegradability. In this case, nutrients concentration after the substrate change remained unchanged therefore there was no need to change the COD:N:P ratio.

A visible difference was observed in gas production in the acidogenic reactor when substrate shifts occurred. In the first shift, there was an increase of 6.4 times in biogas production while in the second shift it decreased by 1.9 times. These differences might be explained by the different influent composition and biodegradability. Raspberry pulp waste has a higher sugar content (especially in the first day after thawing) and a higher SCOD/TCOD ratio, probably promoting an easier fermentation

and resulting in higher gas production. However, the gas composition appeared to remain unchanged after the substrate shift (data not shown) during the period of time represented in Figure 3.9 and Figure 3.10.

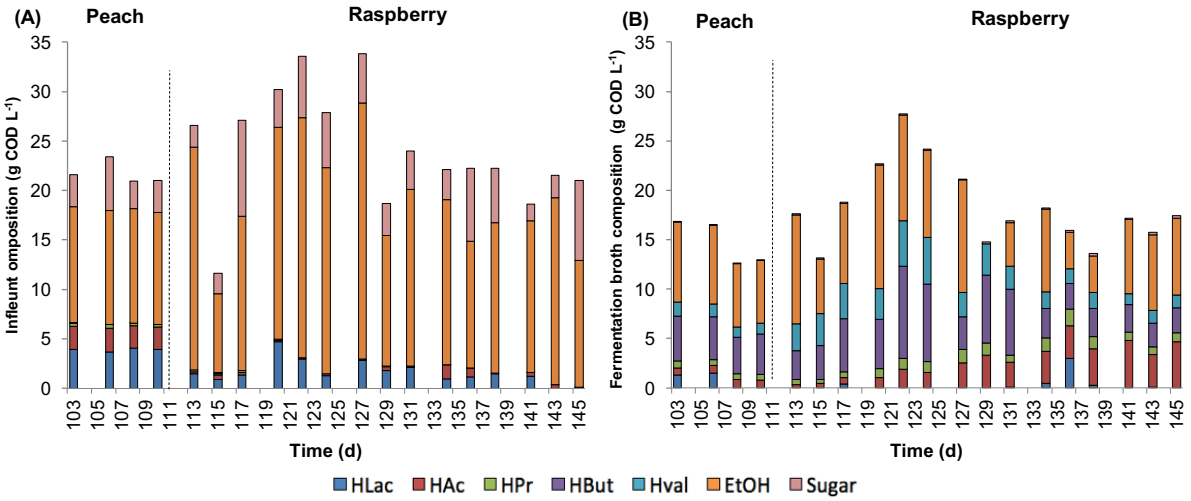

Figure 3.9 Influent (A) and fermentation broth composition (B) in terms of FP and sugar concentration and profiles regarding the first substrate shift: peach to raspberry.

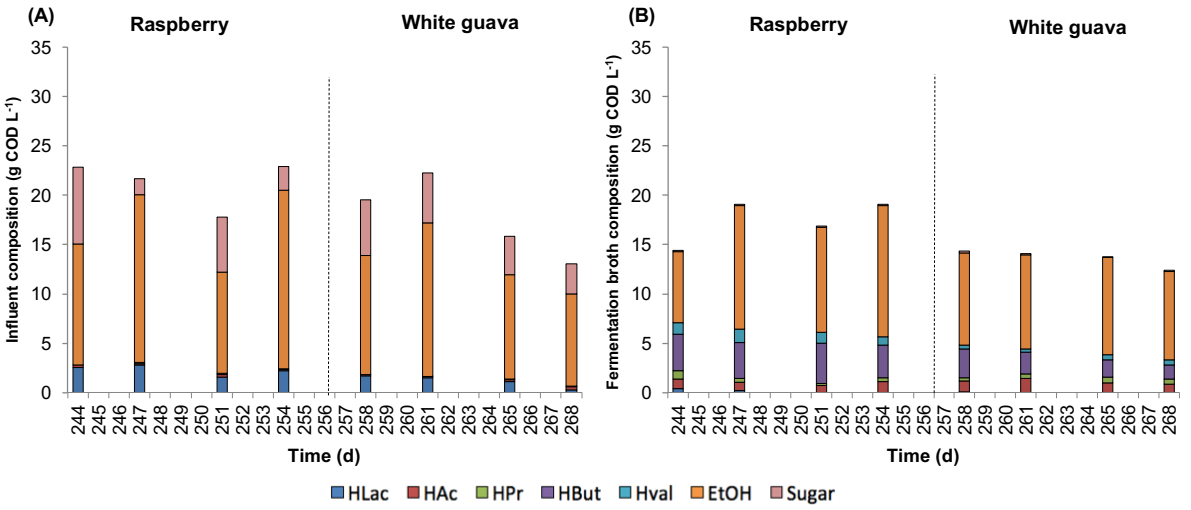

Figure 3.10 Influent (A) and fermentation broth composition (B) in terms of FP and sugar concentration and profiles regarding the second substrate shift: raspberry to white guava.

When the substrate was changed to white guava pulp waste there was a noticeable problem of solids accumulation in the settler. These problems were also noticeable during peach pulp waste fermentation but in a lesser extent and only sporadically during raspberry pulp waste fermentation. Although raspberry pulp waste had the highest TSS concentration among the pulp wastes, raspberry influent had the lowest (2.8 ± 1.1 g L^{-1}). White guava influent had the highest TSS concentration (8.7 ± 0.5 g L^{-1}) followed by peach influent (6.1 ± 1.6 g L^{-1}) (Table 3.2). From these results, it seems that

solids accumulation problems may be associated with the initial concentration of solids in the influent and its biodegradability.

The present results suggest that the reactor could be operated for long periods of time changing the substrate when required without stopping the operation nor needing long periods of time for adaptation. However, considerations in terms of nutrient ratios and solids concentrations should be taken when managing the shift. No studies were found where different monosubstrates were treated sequentially in the same operation for direct comparison with this study. Nevertheless, Fonoll et al., (2015) studied the effect of: (1) shifting from mono-digestion to co-digestion and vice-versa and (2) changing co-substrates (fruit wastes) when treating sewage sludge in a single stage operation. The authors observed that only the change from mono- to co-digestion induced some reactor instability (great increase in VFA concentration) due to tripled OLR values. However, the reactor recovered rapidly. Similarly, in the present study, the instability of FP concentrations in the fermentation broth observed during the initial period after the first substrate shift was associated with the instability of OLR and the reactor was also able to recover rapidly. In the Fonoll et al., (2015) study, VFA concentration was constant when the co-substrate was changed and the operation remained stable. The main difference was regarding CH_4 production which was attributed to the different biodegradability of the fruit wastes. In the present study, constant VFA concentrations and stable operation were also observed when the OLR was maintained during the substrate shift. One of the main differences when changing the substrate was also related with gas production however no specific association with CH_4 production was found.

3.3 Methanogenic operation – Biogas production and overall process efficiency

Regarding the methanogenic reactor performance, this study was mainly focused on two aspects: COD removal and biogas production and composition. COD removal is an indicator of the overall waste treatment efficiency since one of the core goals is the reduction of the organic content present in the wastes. On the other hand, biogas production constitutes one of the most important advantages of using an AD system and its maximisation is a crucial goal. Results regarding these two aspects are summarised in Table 3.5.

Table 3.5 Methanogenic reactor's performance (COD removal and biogas parameters) in different operational conditions. Results regarding start-up conditions are not presented since reactor stabilisation was not reached during that period.

Substrate	Period	Conditions		COD Removal (%)	Biogas production (L d^{-1})	CH_4 yield (L CH_4 g^{-1} COD)	CH_4 productivity (L CH_4 L^{-1} d^{-1})	CH_4 (%)
		HRT (d)	OLR (g COD L^{-1} d^{-1})					
Peach fermentation broth	II	8.6	1.9 ± 0.1	92.8 ± 1.5	3.6 ± 0.8	0.30 ± 0.03	0.54 ± 0.02	77.3 ± 4.3
Raspberry fermentation broth	III	8.6	1.9 ± 0.1	93.2 ± 2.6	3.7 ± 0.8	0.30 ± 0.03	0.56 ± 0.06	75.9 ± 2.8
	IV	5	3.7 ± 0.1	92.5 ± 2.9	6.9 ± 1.0	0.32 ± 0.01	1.08 ± 0.04	75.6 ± 2.0
	V	2.5	7.4 ± 0.6	82.1 ± 3.9	12.8 ± 1.7	0.32 ± 0.05	1.93 ± 0.16	79.1 ± 1.8
White Guava fermentation broth	VI	2	6.8 ± 0.7	85.7 ± 0.5	12.6 ± 1.8	0.37 ± 0.03	2.13 ± 0.13	80.6 ± 0.4

During start-up conditions (peach; HRT of 5 days; OLR of 3.5 g COD L^{-1}), FP were not being consumed effectively leading to a low COD removal (Figure 3.12) and biogas production. Two days after the HRT change to 8.6 days there was already a significant decrease in the FP concentration and SCOD in the reactor (Figure 3.11 and Figure 3.12). The lower OLR and the fact that the substrate was available to be consumed for a longer period of time provided a condition more compatible with the low growth rates of methanogenic microorganisms.

3.3.1 FP removal and COD removal efficiencies

Almost all FP produced in the acidogenic phase were consumed in the methanogenic reactor (Figure 3.11). The increment of OLR led to an initial increase in FP concentration in the effluent, although a constant low FP concentration was achieved in every condition. The most accentuated increase in FP concentration was observed after the change from condition IV to V (OLR from 3.7 to 7.4 g COD L^{-1}). Regardless, the system recovered rapidly achieving a low and stable FP concentration (Figure 3.11 B). The lowest FP concentration in the effluent was verified in condition II (peach; HRT of 8.6 days and OLR of 1.9 g COD L^{-1}). HAc was the main product remaining in the reactor for the most part of the operation with the exception of the start-up (I) and the last two conditions tested (V and VI) as shown in Figure 3.11 B. When HAc is the main FP present in the methanogenic reactor it indicates that the acetogenic activity was efficient avoiding the accumulation of acetate precursors. During start-up conditions, HBut and HVal were present in greater amounts, which suggests a non-efficient acetogenic activity. The accumulation of these VFAs may inhibit methanogenesis. This situation was avoided by changing the HRT/OLR, as referred above. During conditions V and VI, HPr concentration was higher than in the previous conditions probably due to the OLR increase. It is expected that HPr would be the last acetate precursor remaining in the reactor since its conversion is the least thermodynamically favourable. HPr accumulation with concentrations as low as 2.27 g COD L^{-1} has shown to affect methanogenic growth and activity (Li et al., 2012 and references therein). In this case, HPr concentration remained between 0.37 and 0.99 g COD L^{-1} without indication of reactor instability.

COD removal efficiencies in the methanogenic reactor ranged from 82.1 ± 3.9% to 93.2 ± 2.6% (Table 3.5). Bouallagui et al., (2004) obtained COD removal efficiencies in the same range when treating FVW (92.7%) at OLRs between 0.7 – 1.7 g COD L^{-1}, as well as Dareioti et al., (2009) when treating agroindustrial wastes (85.2%) at OLR of 3.5 g COD L^{-1}. Results were also similar to the ones obtained in other studies using two-stage AD systems (Ince, 1998; Diamantis et al., 2014) (Table 3.6). As shown in Table 3.5 and Figure 3.12, COD removal efficiency was similar at HRTs of 8.6 and 5 days (OLR of 1.9 – 3.7 g COD L^{-1} d^{-1}) while it decreased when the HRT was further decreased to 2 – 2.5 days (OLR of 6.8 – 7.4 g COD L^{-1} d^{-1}). In accordance, lower OLRs corresponded to lower SCOD concentration, as shown in Figure 3.12. Therefore, it seems that HRT/OLR changes influences COD removal efficiencies but not in all range. Despite this, SCOD concentration in the reactor stabilised in every condition tested. The lowest SCOD concentration in the reactor was of 0.4 g COD L^{-1} at an OLR of 1.9 g COD L^{-1} d^{-1}, while the average SCOD concentration throughout the stable periods of operation was 1.48 ± 0.77 g COD L^{-1}. These values are still above the Portuguese emission limit value (0.15 g COD L^{-1}) for the discharge of wastewaters in superficial, underground and territorial waters and soil

(Decree-Law no. 236/98). Therefore, a post-treatment strategy may be applied to achieve the limits for discharge. Examples of post-treatment strategies include physico-chemical treatment, facultative ponds and constructed wetlands (van Lier et al., 2008). Another strategy is to recirculate the methanogenic effluent into the acidogenic reactor reducing the amount of effluent to be discharged. The methanogenic effluent would be used to dilute the pulp waste for the acidogenic influent preparation, reducing or eliminating the need to use tap water. This type of recirculation has shown to increase both H_2 and CH_4 production (O-Thong et al., 2016) and reduce the use of reagent for pH adjustment in the acidogenic reactor due to an enhanced alkalinity (Ganesh et al., 2014 and references therein). Nonetheless, the effluent could be transferred to municipal wastewater treatment plants since this low COD and solids concentration would not be expected to cause problems in its operation.

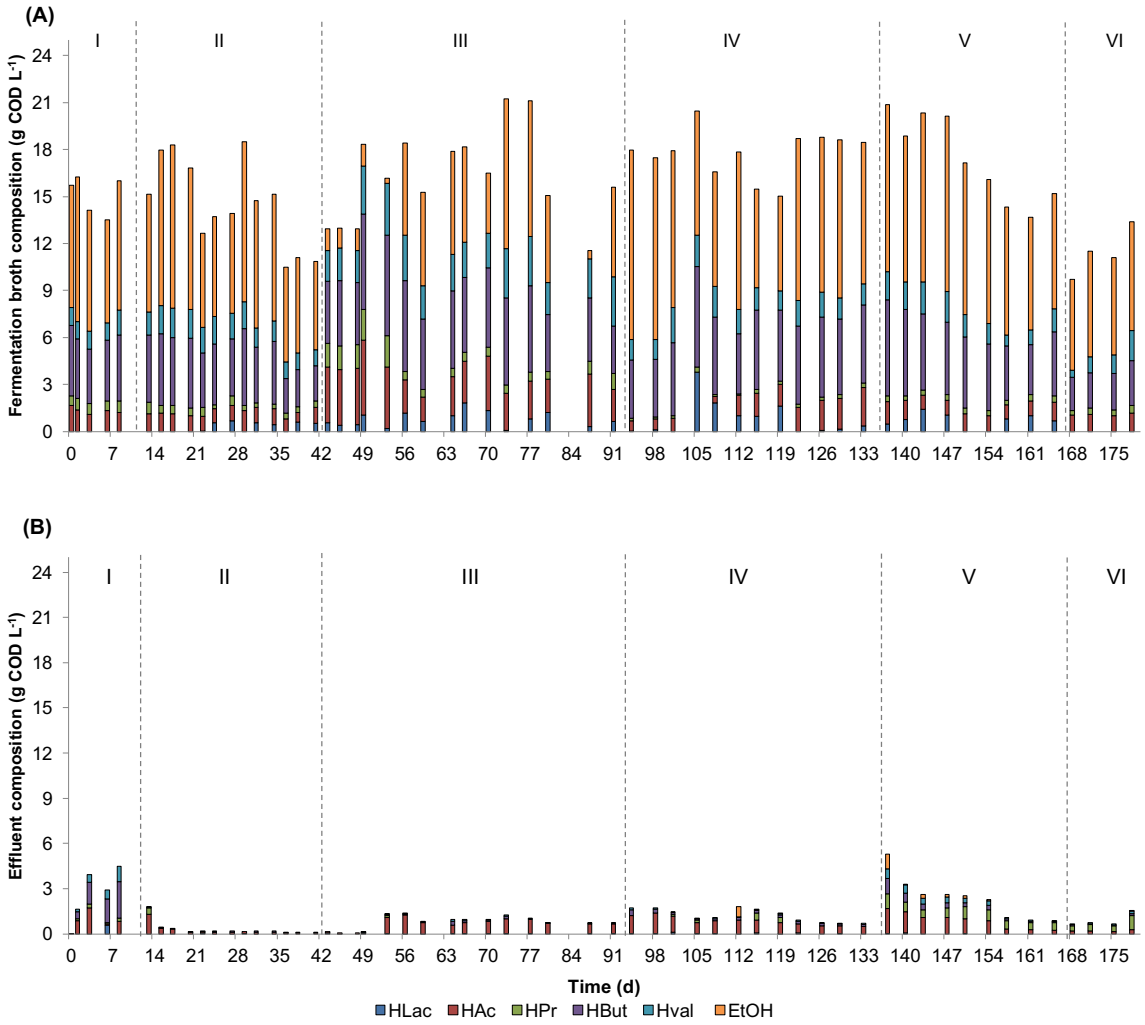

Figure 3.11 Fermentation broth (A) and effluent (B) FP concentration and profiles in the methanogenic reactor during the 178 days of operation under different operational contidions: (I) Peach, HRT 5 d, OLR 3.5 ± 0.1 g COD L^{-1} d^{-1}; (II) Peach, HRT 8.6 d, OLR 1.9 ± 0.1 g COD L^{-1} d^{-1}; (III) Raspberry, HRT 8.6 d, OLR 1.9 ± 0.1 g COD L^{-1} d^{-1}; (IV) Raspberry, HRT 5 d, OLR 3.7 ± 0.1 g COD L^{-1} d^{-1}; (V) Raspberry, HRT 2.5 d, OLR 7.4 ± 0.6 g COD L^{-1} d^{-1}; (VI) White guava, HRT 2d, OLR 6.8 ± 0.7 g COD L^{-1} d^{-1}.

Similar to the acidogenic reactor, it was also possible to assess the effect of changing substrates on FP concentration and profiles in the methanogenic reactor. Peach and raspberry fermentation broths were both treated in the same condition of OLR of 1.9 g COD L^{-1} d^{-1} and HRT of 8.6 days (Conditions II and III). Although the FP profiles of these fermentation broths were slightly different, with higher HAc concentration in the raspberry fermentation broth (Figure 3.11 A), similar COD removal efficiencies were obtained (Figure 3.12). This suggests that the microbial community in the granules was diverse enough to metabolise different ratios of FP and remained equally active after the substrate changed. Nevertheless, a lower FP concentration in the effluent was obtained from the peach fermentation broth (Figure 3.11 B). Regarding the change from raspberry to white guava fermentation broth, the HRT was changed from 2.5 to 2 days in order to maintain the OLR since the white guava fermentation broth had a lower SCOD concentration. Nevertheless, after the substrate shift, the FP concentrations and profile were immediately constant and similar to the ones observed before the shift (Figure 3.11 B). COD removal efficiencies were also similar between the two conditions. This shows that it was possible to maintain a stable operation of the methanogenic reactor using fermentation broths from different fruit pulp wastes fermentations in the acidogenic stage.

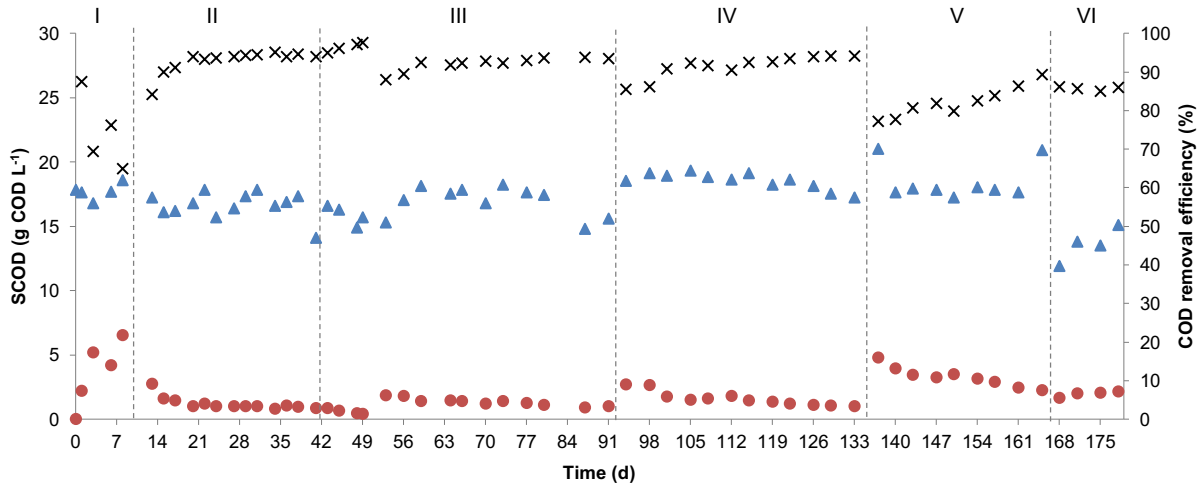

Figure 3.12 COD variation in the fermentation broth (▲) and reactor (●) and COD removal efficiency (×) during 178 days of operation under different operational contidions: (I) Peach, HRT 5 d, OLR 3.5 ± 0.1 g COD L^{-1} d^{-1}; (II) Peach, HRT 8.6 d, OLR 1.9 ± 0.1 g COD L^{-1} d^{-1}; (III) Raspberry, HRT 8.6 d, OLR 1.9 ± 0.1 g COD L^{-1} d^{-1}; (IV) Raspberry, HRT 5 d, OLR 3.7 ± 0.1 g COD L^{-1} d^{-1}; (V) Raspberry, HRT 2.5 d, OLR 7.4 ± 0.6 g COD L^{-1} d^{-1}; (VI) White guava, HRT 2d, OLR 6.8 ± 0.7 g COD L^{-1} d^{-1}.

Although the main conversions in the methanogenic reactor are acetogenesis and methanogenesis, it was possible to observe sugar and protein removals of 81.1 ± 15.2% and 63.4 ± 7.8%, respectively. The global sugar concentration in the fermentation broth was of 0.10 ± 0.04 g L^{-1} while in the effluent it was of 0.01 ± 0.01 g L^{-1}. The global protein concentration was of 0.37 ± 0.08 g L^{-1} in the fermentation broth and of 0.14 ± 0.02 g L^{-1} in the effluent. This suggests the presence of active fermentative bacteria in the methanogenic reactor, probably located in the outer layer of granules (Fang et al., 1994; Lim and Kim, 2014 and references therein).

These results show that a two-stage configuration allowed the stable operation of the methanogenic reactor with consecutive OLR increments up to 7.4 g COD L^{-1} without compromising the reactor stability and achieving low FP concentrations and high COD removal efficiencies. Although COD removal decreased with increasing OLR, the values obtained at the highest OLR were still similar or superior to other studies where higher HRTs and lower OLRs were applied (Bouallagui et al., 2004; Paudel et al., 2017) (Table 3.6).

3.3.2 Biogas production and composition

The average biogas production varied between 3.6 ± 0.8 – 12.8 ± 1.7 L d^{-1} (Table 3.5). Biogas production increased as HRT decreased/OLR increased, regardless of the substrate used, as shown in Figure 3.13, which is coherent with the high COD removal efficiencies obtained. Although the FP profiles of the fermentation broth are not completely constant (Figure 3.11 A), this did not seem to affect biogas production nor biogas composition, suggesting a stable activity of the archaea enriched microbial community of the granules. Instead, biogas composition remained fairly stable throughout the conditions tested as shown in Figure 3.14. Therefore, although the different substrates led to different gas production rates in the acidogenic reactor, this was not observed in the methanogenic reactor. H_2 was never detected in the methanogenic reactor which suggests that its rate of removal to produce CH_4 was evenly paired with its production rate showing effective and unperturbed syntrophic relationships. The average CH_4 content varied between 75.6 ± 2.0% and 80.6 ± 0.4% (Table 3.5) and no evident differences were found between conditions. These CH_4 content results were among the highest observed in studies on two-stage AD systems treating FW or FVW, as shown in Table 3.6. Usually, two-stage systems produce biogas with higher CH_4 content compared to one-stage systems (Voelklein et al., 2016). Indeed, Scano et al., (2014) observed average CH_4 contents between 50 – 60% at low OLRs between 2.5 – 3.0 g VS d^{-1} L^{-1} when treating FVWs in a single stage system. The separate digestion of three components (seeds, pulps and peel) of different fresh and rotten fruits was carried out by Sanjaya et al., (2016) in single stage batch operations to evaluate the potential for CH_4 production. The biogas produced in that study contained between 52 – 62% of CH_4, which was considerably lower than in the present study where similar wastes (fruit pulp) were used.

CH_4 productivity increased as HRT decreased/OLR increased concomitantly with the increase in gas production. CH_4 productivity varied from 0.54 ± 0.02 to 2.13 ± 0.13 L CH_4 L d^{-1}. Bouallagui et al., (2004) reported a CH_4 productivity of 0.52 L CH_4 L d^{-1} when treating FVW at an OLR of 1.65 g COD L^{-1} d^{-1} and HRT of 10 days which is similar to the result obtained in this study at an OLR of 1.9 g COD L^{-1} d^{-1} and HRT of 8.6 days (0.54 ± 0.02 and 0.56 ± 0.06 L CH_4 L d^{-1}, for peach and raspberry fermentation broths, respectively). The CH_4 yields obtained in this study were similar to the theoretical value of 0.35 L CH_4 g^{-1} COD (van Lier et al., 2008) and did not vary greatly between conditions (0.30 ± 0.03 – 0.37 ± 0.03 L CH_4 g^{-1} COD) as shown in Figure 3.13 and Table 3.5. The CH_4 yields obtained in this study are among the highest obtained in the studies presented in Table 3.6. In the present study, there was no evident association between HRT/OLR changes and CH_4 yield and content. Voelklein et al., (2016) also found no correlation between CH_4 yield and OLR increments (at constant HRT)

between 2.9 and 7.3 g COD L^{-1} d^{-1}. On the other hand, Paudel et al., (2017) observed a decrease in CH_4 yield and content due to the impaired growth of methanogens when the HRT decreased from 20 to 15 days (OLR from 1.24 to 1.76 g VS L^{-1} d^{-1}). Still, the OLR range used in that study was small and only two conditions were tested. It would be interesting to confirm if that behaviour would remain in a broader range of HRT/OLR.

Energy recovery considering only the CH_4 produced in the methanogenic reactor remained fairly stable at 12.04 ± 1.58 kJ g^{-1} COD throughout the time of operation as shown in Figure 3.13. Voelklein et al., (2016) obtained slightly lower energy recovery values between 9.15 – 10.33 kJ g^{-1} COD at similar OLRs (2.9 – 7.3 g COD L^{-1}) but higher HRT (12 d). When operating at the lowest HRT (2 d), the CH_4 produced in this study could generate energy up to 432.8 kJ d^{-1}. Energy recovery can be become even higher in two-stage systems when H_2 production in the acidogenic stage is considered in its calculation (Fu et al., 2017). Previous studies have reported 11 – 19% higher energy yields in two-stage systems due to H_2 production when compared to single stage operations (Fu et al., 2017; Luo et al., 2011; Nasr et al., 2012; Nathao et al., 2013). Nonetheless, the acidogenic phase may be solely considered as a pre-treatment and partial upgrading system which contributes for the production of a biogas richer in CH_4, decreasing the costs on biogas upgrading (Voelklein et al., 2016).

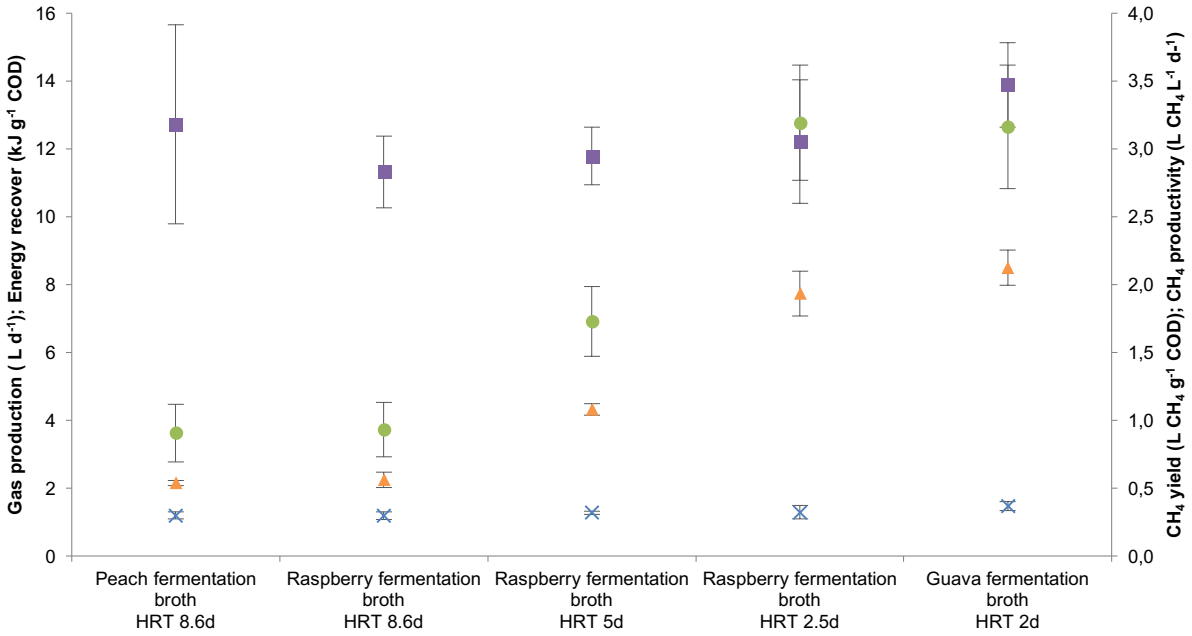

Figure 3.13 Biogas production parameters in each condition tested: Gas production (●), CH_4 yield (×), CH_4 productivity (▲) and energy recovery (■). Error bars represent one standard deviation. Results regarding start-up conditions are not presented since reactor stabilisation was not reached during that period.

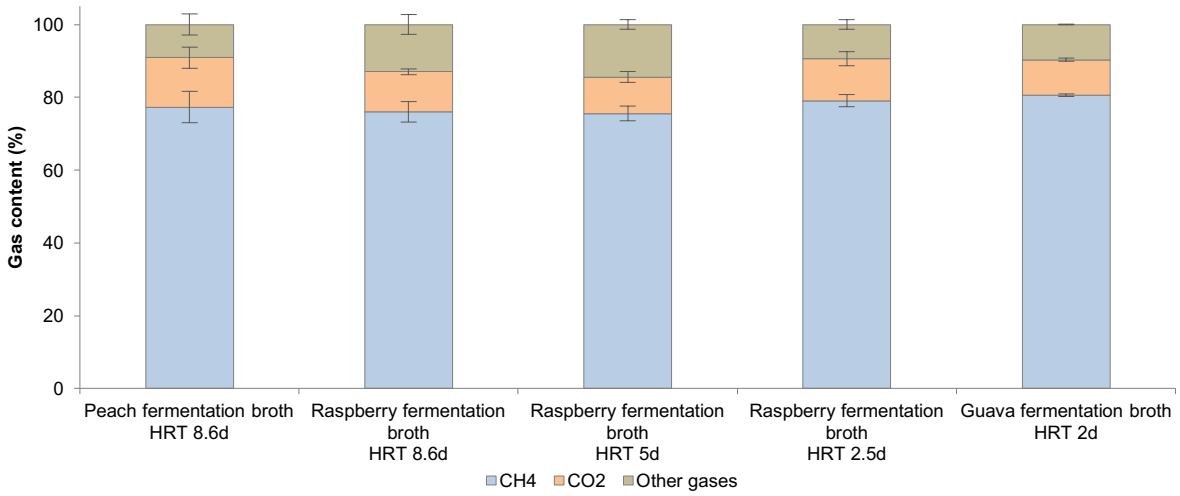

Figure 3.14 Average biogas composition produced in the methanogenic reactor in each condition tested, in chronological order. "Other gases" is refered to N_2 and O_2. Error bars represent one standard deviation.

As referred in the Materials and methods section, regarding the methanogenic reactor operation, only one pH value was tested: 7.5. However, on the 177th day of operation there was a problem with the pH controller which led to the decrease of pH to 7.05 during approximately 18 hours. The pH was maintained neutral showing a good buffer capacity in the reactor. It was possible to observe a change in the biogas composition associated with this pH decrease. CH_4 decreased from 80% to 65% and CO_2 increased from 10% to 25%. Moreover, there was a slight increase in the average gas flow from around 575 mL/h to approximately 685 mL/h. Once the pH was re-established to 7.5, gas flow and gas composition returned to their previous values in a few hours. These changes in biogas production and composition might have been caused by the release of carbon species in the form of CO_2 which were dissolved at a higher pH or, on the other hand, it can also suggest that a slight decrease in pH may have a considerable effect on the microorganisms' metabolism. Regardless, the system was able to recover right after the previous conditions were re-established which indicates a fine robustness.

Choosing the best operational conditions among the ones evaluated in the present work depends on a balance between COD removal and biogas/CH_4 production. If the main goal is to reduce the organic matter to its minimum possible, it can be advantageous to operate at higher HRTs (lower OLRs). However, as the acidogenic reactor was operated at lower HRTs, the reactors operations would not be coordinated and there would be accumulation of acidogenic fermentation broth to be treated. This problem can be solved by using a methanogenic reactor with larger volumes, which is applied in pilot scale systems (Grimberg et al., 2015; Sen et al., 2016 and references therein). On the other hand, CH_4 production is one of the biggest advantages of using AD and smaller HRTs (higher OLRs) provides higher CH_4 productivities due to overall higher gas production. In the specific case of this work, as the main goal was to maximise CH_4 production and both reactors have the same working volume, operating the methanogenic reactor at lower HRTs (2 – 2.5 days) and higher OLRs appears to be beneficial considering that the operation remains stable which allows the treatment of a higher amount of fermentation broth in the same period of time. As the resultant SCOD in these conditions is of around 2 g COD L^{-1}, the effluent may be subjected to a post-treatment as referred above.

Table 3.6 Examples of the use of two-stage anaerobic digestion systems to treat real wastes.

Substrate	pH		OLR		HRT (d)		Acidification degree (%)	Organic matter removal (%)	CH_4 productivity (L CH_4 L^{-1} d^{-1})	CH_4 yield	CH_4 (%)	Reference
	RA	RM	RA	RM	RA	RM						
Food waste and brown water CoAD[a]	5.0 – 5.5	7.0 – 7.5	17.7 – 106 (g VS L^{-1} d^{-1})	1.2; 1.8 (g VS L^{-1} d^{-1})	0.3 – 2.0	20.0; 15.0	-	62.0; 50.4 (COD)	0.82; 0.77	0.22; 0.18 (L CH_4 g^{-1} COD)	70; 60	Paudel et al., 2017
Food waste[a]	5.5	7.5 – 7.9	8.8 – 21.9 (g COD L^{-1} d^{-1})	2.9 – 7.3 (g COD L^{-1} d^{-1})	4.0	12.0	64.1 – 88.5[1]	-	-	0.25 – 0.29 (L CH_4 g^{-1} COD)	67 – 74	Voelklein et al., 2016
Waste activated sludge[b]	6.8	8.2	18.0 (g COD L^{-1} d^{-1})	2.6 (g COD L^{-1} d^{-1})	2.0	18.0; 20.0	-	38.0 (VS)	0.38	0.17 (L CH_4 g^{-1} COD_{fed})	69	Leite et al., 2016
FVW[a]	4.5 – 5.8	7.4 – 7.8	5.8 – 11.6 (g COD L^{-1} d^{-1})	1.2 – 1.4 (g COD L^{-1} d^{-1})	5.0 – 10.0	-	-	98.0 (COD)	-	0. 26 (L CH_4 g^{-1} COD)	73 – 80	Ganesh et al., 2014
Cheese whey[a]	4.0 – 5.0	6.4	22.2 (g COD L^{-1} d^{-1})	6.7 – 23.4 (g COD L^{-1} d^{-1})	0.4	0.5 – 1.2	-	78.4 – 87.6 (COD)	< 7	0.31 – 0.37 (Nl CH_4 g^{-1} COD)	57 – 59	Diamantis et al., 2014
Food waste recycling wastewater[a]	< 4.5	7.6 – 8.1	5.7 – 35.6 (RA + RM) (g COD L^{-1} d^{-1})		0.7 – 4.2	3.3 – 20.8	-	71.0 – 82.3 (COD)	< 8	27.1 – 42.2 (L CH_4 L^{-1} substrate)	-	Shin et al., 2010
FVW[a]	5.5	6.9 –7.5	3.7 – 10.0 (g COD L^{-1} d^{-1})	0.7 - 1.7 (g COD L^{-1} d^{-1})	3.0	10.0	38.9 – 44.4[2]	67.9 – 92.7 (COD)	0.18 – 0.52	0.32 (L CH_4 g^{-1} COD_{fed})	69 – 71	Bouallagui et al., 2004
Dairy wastewater[a]	5.5 – 6.0	7.0 – 7.5	< 23.0 (g COD L^{-1} d^{-1})	< 7.0 (g COD L^{-1} d^{-1})	0.5	1.5	< 61.0	> 85.0 (COD)	-	-	75 – 80	Ince, 1998
Fruit pulp waste[a]	4.5 – 5.5	7.5	10.7 – 25.7 (g COD L^{-1} d^{-1})	1.9 – 7.4 (g COD L^{-1} d^{-1})	1.0 – 2.0	2.0 – 8.6	53.7 – 76.4[1]	82.1 – 93.2 (COD)	0.54 – 2.13	0.30 – 0.37 (L CH_4 g^{-1} COD)	76 - 81	This work

Notes: [a]mesophilic conditions; [b]thermophilic conditions; acidification degree considering [1]total FP or [2]total VFA

Abbreviations: RA – acidogenic reactor; RM – methanogenic reactor

3.3.3 Nutrients concentration and biomass composition

PO_4-P and NH_4-N concentrations were measured to assess the microbial growth and nutrient limitation, as in the acidogenic reactor. There was no indication of nutrient limitation throughout the operation showing that the nutrients present in the acidogenic fermentation broth were enough to supply methanogens. PO_4-P concentration in the effluent remained constantly lower than its concentration in the fermentation broth throughout time as shown in Figure 3.15 (A). This was also verified for NH_4-N in the last three conditions tested (IV, V, VI), as shown in Figure 3.15 (B). The transition from condition II to III corresponded to the change from peach to raspberry fermentation broth. Raspberry fermentation broth had considerably lower NH_4-N concentration than peach's. This explains the abrupt decrease in NH_4-N concentration in the fermentation broth and the correspondent gradual decrease in NH_4-N concentration in the effluent. These results suggest a good biomass stability where cell growth outweighed cell lysis.

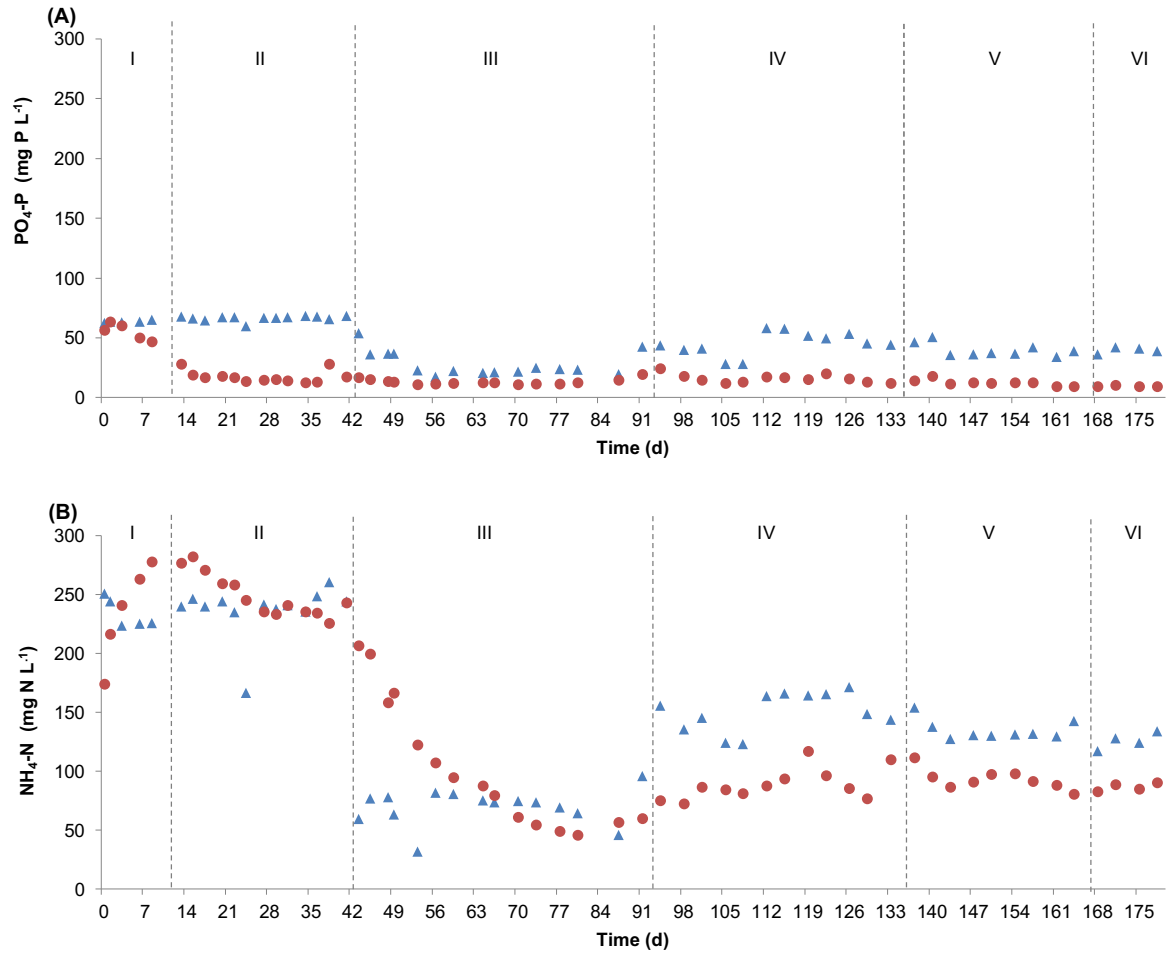

Figure 3.15 (A) Phosphorus and (B) ammonium concentrations in the fermentation broth (▲) and in the effluent (●) during the 178 days of operation under different operational contidions: (I) Peach, HRT 5 d, OLR 3.5 ± 0.1 g COD L^{-1} d^{-1}; (II) Peach, HRT 8.6 d, OLR 1.9 ± 0.1 g COD L^{-1} d^{-1}; (III) Raspberry, HRT 8.6 d, OLR 1.9 ± 0.1 g COD L^{-1} d^{-1}; (IV) Raspberry, HRT 5 d, OLR 3.7 ± 0.1 g COD L^{-1} d^{-1}; (V) Raspberry, HRT 2.5 d, OLR 7.4 ± 0.6 g COD L^{-1} d^{-1}; (VI) White guava, HRT 2d, OLR 6.8 ± 0.7 g COD L^{-1} d^{-1}.

Biomass concentration is an important aspect that directly impacts waste degradation and biogas production. During the methanogenic reactor operation, a decrease in granules size comparing to the inoculum was observed probably due to the system being mechanically agitated. However, granules maintained their macroscopic appearance throughout the operation. VSS analysis was performed in order to monitor biomass concentration and possible biomass washout. The average VSS concentration remained constant throughout all conditions tested (Figure 3.16). As expected, granules were mainly located at the bottom of the reactor which indicates good settling properties. Over time, there is a slight decrease in VSS concentration in the three upper heights (h1, h2, h3). Some of the biomass in those heights might have suffered washout at lower HRTs or granules could have become successively heavier, and settling at the bottom of the reactor.

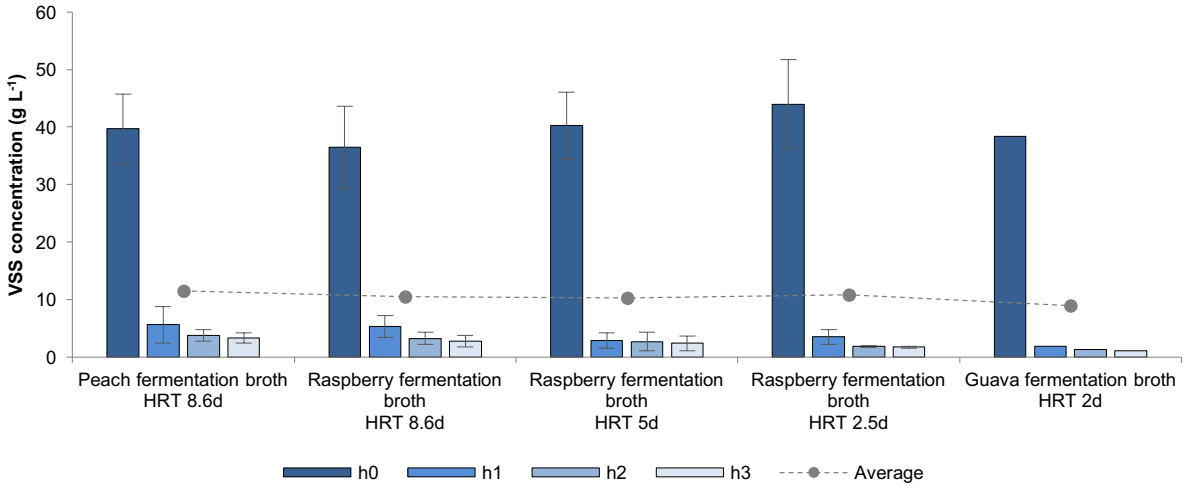

Figure 3.16 VSS concentration in the methanogenic reactor at different heights from the bottom of the reactor: h0 (0 cm); h1 (10 cm); h2 (17 cm) and h3 (25 cm) (conditions are presented in chronological order). Error bars represent one standard deviation. The absence of error bars indicates that the average presented was calculated based on < 3 samples.

FISH was performed in order to obtain a qualitative analysis of the archaea community (Table 3.7). *Methanosaeta* and *Methanosarcina* are acetoclastic methanogens while Methanomicrobiales and Methanobacteriales are hydrogenothrophic methanogens. The microbial community in the methanogenic reactor was predominantly composed of archaea microorganisms enriched in *Methanosaeta* with the constant presence of Methanobacteriales (Table 3.7). Samples were only taken at the beginning and at the end of each condition, therefore it was not possible to conclude about the changes in microbial community throughout each condition. There was no apparent relation between OLR/HRT changes and the relative composition of the archaea community since the last seemed to have remained constant throughout time. Luo et al., (2011) also found the relative abundance of archaea community in methanogenic reactors to be independent of substrate and HRT. However, in that study, *Methanosarcina* organisms were dominant. Karakashev et al., (2005) also found no apparent influence of OLR on the dominant methanogens observed. In that study, *Methanosaetaceae* was the dominant family at low HOrgs (< 1 g HAc eq L^{-1}) and ammonium concentrations (1.5 g N L^{-1}) in mesophilic reactors, which is in accordance with the present study. Nevertheless, literature is limited and sometimes contradictory regarding the specific effects of pH,

OLR, VFA concentration and substrate composition on the community's composition and dynamics (Ferguson et al., 2016 and references therein).

Table 3.7 Microbial community in the methanogenic reactor – qualitative analysis based on FISH.

Day	Substrate	Conditions			Probes (microorganisms)				
		pH	HRT	Height	ARC915 (Archaea)	MX825 (*Methanosaeta*)	MG-1200b (Methanomicrobiales)	MB1174 (Methanobacteriales)	MS821 (*Methanosarcina*)
1	Inoculation	7.5	5	h0	+ +	+ +	+ +	+	+
				h3	+ +	+ +	+ –	+	+ –
29 (middle)	Peach fermentation broth	7.5	8.6	h0	+ + +	+ + +	+ +	+	+ +
				h3	+ + +	+ +	+ –	+	+
41 (end)	Peach fermentation broth	7.5	8.6	h0	+ + +	+ + +	+	+	+
				h3	+ + +	+ +	+ –	+	+
92 (end)	Raspberry fermentation broth	7.5	8.6	h0	+ + +	+ + +	+	+	+ +
				h3	+ + +	+ +	+ –	+	+
135 (end)	Raspberry fermentation broth	7.5	5	h0	+ + +	+ + +	+	+	+ –
				h3	+ +	+ + +	+ –	+ +	+ –
164 (end)	Raspberry fermentation broth	7.5	2.5	h0	+ + +	+ + +	+	+ +	+
				h3	+ +	+ + +	+ –	+	+ –
178 (end)	White guava fermentation broth	7.5	2	h0	+ + +	+ + +	+ +	+	+
				h3	+ +	+ + +	+ –	+ –	+ –

(–) Non or almost non-existant; (+ –) Identifiied (1 – 5%); (+) Present (5 – 20%); (++) Abundant (20 – 50%); (+++) Extremely abundant (> 50%)
(middle), (end) refers to each condition time frame

3.4 Biogas upgrading – Performance assessment of MMMs with MOFs on CH_4 and CO_2 separation

Biogas upgrading is an essential step in order to remove impurities and achieve the CH_4 content required for several applications such as natural gas or vehicle fuel. This part of the present work is focused on preliminary studies on the preparation and characterisation of MMMs with MOFs and the evaluation of their performance on CH_4 and CO_2 separation.

3.4.1 MOF-5 characterisation

Two different MOFs (MIL-53 and MOF-5) were used as inorganic fillers in a polymeric matrix (Matrimid®5218 matrix) for MMMs production. These MOFs were chosen based on promising results in CH_4/CO_2 separation obtained in previous studies (Feijani et al., 2015; Basu et al., 2011 and references therein; Dosrati et al., 2014). It has been reported that CO_2 interacts strongly with the hydroxyl groups present on MIL-53 promoting a good separation towards this gas (Basu et al., 2011). While MIL-53 was used as it was received, MOF-5 was synthesised during this work. XRD pattern of MOF-5 is shown in Figure 3.17 where the MOF crystallinity is confirmed due to the sharpened appearance of the peaks. The presence of residual water can induce a framework distortion creating a new phase which is shown by the concomitant appearance of a peak at 8.9° and the disappearance of peaks at 6.9º and 9.7º (Ming et al., 2015; Monteiro et al., 2017) as it is shown in Figure 3.17.

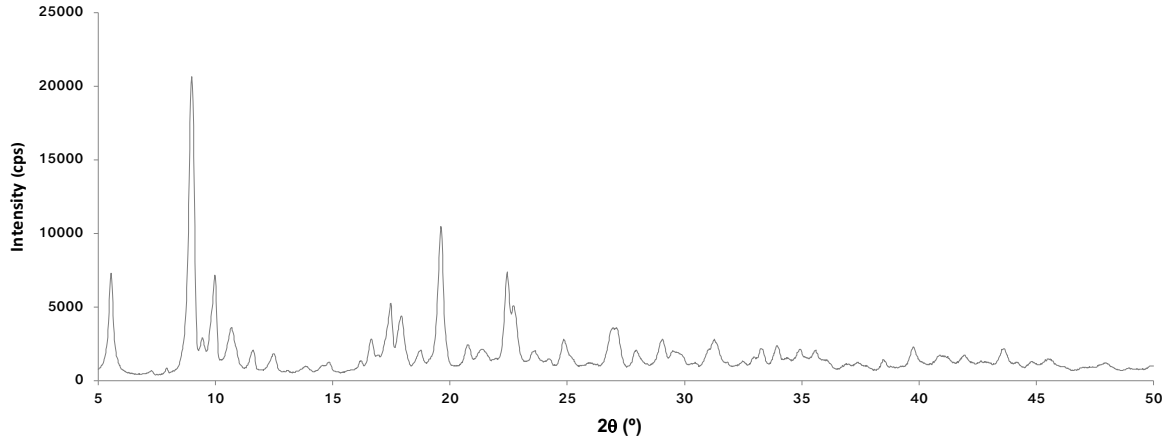

Figure 3.17 XRD pattern of the synthesised MOF-5.

3.4.2 MMMs characterisation and performance on single gas permeation studies

MMMs using three MOF loadings ($w_{MOF}/w_{Matrimid}$): 10% 20% and 30% were successfully prepared. Furthermore, a polymeric membrane of Matrimid®5218 was also prepared for results comparison, being considered the membrane with 0% MOF loading. In order to evaluate the membranes' hydrophilic/hydrophobic nature, contact angles (θ) were measured and the results are shown in Figure 3.18. The contact angle corresponds to the angle formed by the intersection of the liquid-solid interface and the liquid-vapour interface. Each solid-liquid system in a specific environment has, in theory, a characteristic contact angle. When using water, if the angle is smaller than 90°, the

membrane's surface is considered hydrophilic while if the angle is higher than 90°, it is hydrophobic (Yuan and Lee, 2013). The polymeric membrane composed by Matrimid®5218 is hydrophilic with a contact angle of 81.8°, which was in accordance with previous studies (Nabais, 2016; Nayak, 2013). The addition of both MOF-5 and MIL-53 improved the membrane hydrophilicity for all the MOF loadings tested (each θ < 77°). It was not possible to correlate the contact angle with the changes in MOF loading.

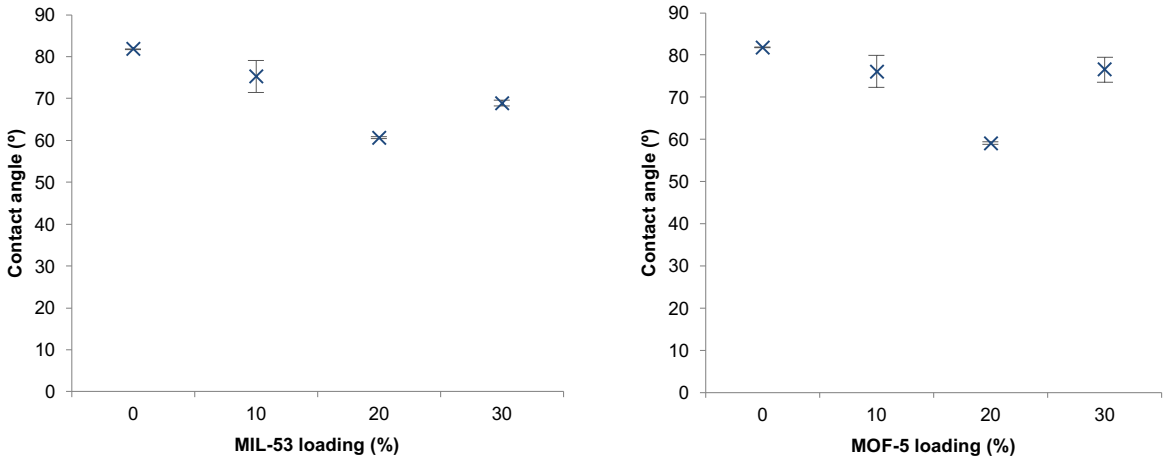

Figure 3.18 Contact angles of the membranes (A) Matrimid®5218+MIL-53 (B) Matrimid®5218+MOF-5. Error bars correspond to the standard error.

Single gas permeation studies were performed in order to evaluate the CO_2 permeability (PCO_2) and ideal selectivity ($\alpha CO_2/CH_4$). In order to achieve the most efficient CO_2/CH_4 separation, these parameters should be maximised. The duration of the experiment was similar for each membrane. The results are summarised in Table 3.8.

Table 3.8 Permeability and selectivity of the membranes synthesised using different MOF loadings

Membrane composition	Permeability (m^2 s^{-1})		Permeability (barrer)		Ideal selectivity
	CH_4	CO_2	CH_4	CO_2	α CO_2/CH_4
Matrimid®5218	$9.38x10^{-13}$	$7.98x10^{-12}$	1.13	9.62	8.51
Matrimid®5218 + 10% MIL-53	$4.49x10^{-13}$	$3.70x10^{-11}$	0.54	44.58	82.56
Matrimid®5218 + 20% MIL-53	$6.11x10^{-13}$	$2.31x10^{-11}$	0.74	27.83	37.61
Matrimid®5218 + 30% MIL-53	$2.00x10^{-12}$	$2.63x10^{-11}$	2.41	31.69	13.15
Matrimid®5218 + 10% MOF-5	$1.59x10^{-12}$	$6.85x10^{-11}$	1.92	82.53	42.98
Matrimid®5218 + 20% MOF-5	$1.92x10^{-12}$	$7.51x10^{-11}$	2.32	90.48	39.00
Matrimid®5218 + 30% MOF-5	$1.35x10^{-12}$	$2.54x10^{-11}$	1.63	30.65	18.80

1 Barrer = $8.3x10^{-13}$ m^2 s^{-1}

Concerning Matrimid®5218+MIL-53 membranes, both PCO_2 and $\alpha CO_2/CH_4$ are superior when compared with Matrimid®5218 suggesting a good adhesion and compatibility between the polymer and the MOF particles. The Matrimid®5218+10%MIL-53 (w/w) membrane presented the highest PCO_2 value (44.58 Barrer) and $\alpha CO_2/CH_4$ (82.56) suggesting this is the membrane with better separation properties. Therefore, it seems that the increment of MOF loading has a negative impact on PCO_2 and selectivity, at least in the range studied (10 – 30%) (Figure 3.19 and Figure 3.20). CH_4 permeability increased as MOF loading increased suffering a sharp increase at 30% (w/w) loading. This tendency was also observed at by Dorosti et al., (2014) when studying membranes with the same composition,

although the sharp increase on CH_4 permeability occurred at 20% (w/w) loading. Higher MOF loadings may have promoted the formation of voids at the interface of particles and the polymer and promote stress due to an increase of rigidity and the tendency of the particles to agglomerate, as suggested by Car et al., (2006) and observed by Dorosti et al., (2014). In order to confirm this theory, it would be necessary to perform further membrane characterisation studies such as Scanning Electron Microscopy (SEM), Energy-dispersive X-ray spectroscopy mapping (EDS), as well as the evaluation of the mechanical properties. Conversely, Basu et al., (2011) synthesised membranes with the same composition as the ones in the present study, and observed an increase in both PCO_2 and selectivity with increasing MOF loadings. In this study, membrane selectivity for binary gas mixture CO_2/CH_4 (35/65%) varied between ≈ 27 and 33. Dorosti et al., (2014) observed increasing performances with MOF loading increase up until 15% loading. However, the performance of the MMMs with 20% MOF loading was severely inferior.

CO_2 permeability and ideal selectivity CO_2/CH_4 of the Matrimid®5218+MOF-5 membranes were also superior when compared with Matrimid®5218. Both CO_2 and CH_4 permeabilities increased with an increase in MOF loading until 20% loading, while obtaining similar selectivities. The lowest CO_2 and CH_4 permeabilities and selectivity values obtained among the Matrimid®5218+MOF-5 membranes corresponded to the 30% (w/w) loading. Again, this might be associated with MOF particles aggregation as referred to Matrimid®5218+MIL-53 membranes. Perez et al., (2009) studied the performance of Matrimid® based MMM with MOF-5 (0%, 10%, 20%, 30% loading) in the separation of various single gases and gas mixtures. The permeability of all gases, including CH_4 and CO_2, increased with MOF loading while the selectivities remained constant. In terms of selectivity, these results are in agreement with the presented results with the exception of the Matrimid®+30%MOF-5 (w/w) membrane. In order to increase the confidence in the results presented in the present work, membrane synthesis and gas permeation studies should be performed in replicate. It could also be interesting to test intermediary MOF loadings.

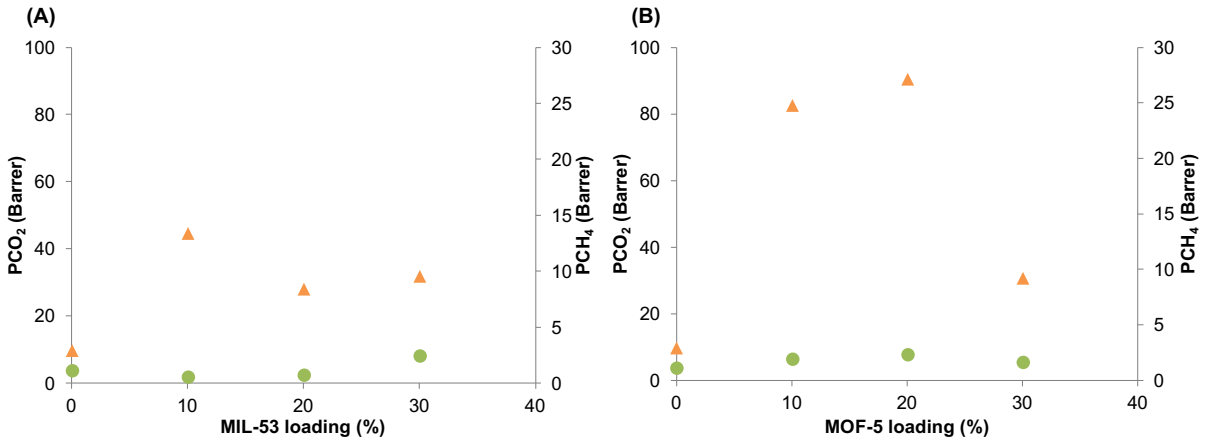

Figure 3.19 CO_2 (▲) and CH_4 (●) permeabilities in membranes with increasing MOF loadings, at 30 ºC. (A) Results regarding Matrimid® 5128+MIL-53 membranes; (B) Results regarding Matrimid® 5128+MOF-5 membranes.

In order to compare the results obtained in this work with those available in the literature, the CO_2/CH_4 ideal selectivity was represented as a function of PCO_2 (as illustrated in Figure 3.20). In the same representation, the Robeson upper bound correlation was plotted. The Robeson's upper bound was determined based in a vast selection of gas separation studies with polymeric membranes and represents the trade-off relationship, where $\alpha CO_2/CH_4$ tends to decrease as PCO_2 increases (Robeson, 2008). When fabricating new membranes, there is the goal to achieve a good compromise between permeability and selectivity in order to surpass the empirical upper bound. In this work, it has been observed that Matrimid®5218 with 10% (w/w) MIL-53 membrane overlaps the Robeson's upper bound which indicates a better performance when compared with literature. Matrimid®5218 with MOF-5 (10 and 20% loadings) are also very close to the upper bound. The remaining membranes tested in this study are below the Robeson's upper bound showing no significant improvement in selectivity and permeability compared with previous studies.

Since the highest PCO_2 (Matrimid®+20%MOF-5) did not correspond to the membrane with higher $\alpha CO_2/CH_4$ (Matrimid®+10%MIL-53), the choice of the most adequate membrane for commercial application may not be straightforward. Concerning this topic, Havas and Lin (2017) performed a process simulation and techno-economic analysis on membranes for CO_2 removal in biogas upgrading. Simulations took into consideration the trade-off between permeability and selectivity. It was observed that, in one-step membrane systems, the membrane area required as well as operational and capital costs increases as selectivity increases (or permeability decreases). On the other hand, CH_4 losses are reduced. The authors concluded that optimal membranes for biogas upgrading should have high CO_2 permeability and sufficient selectivity to achieve minimal costs.

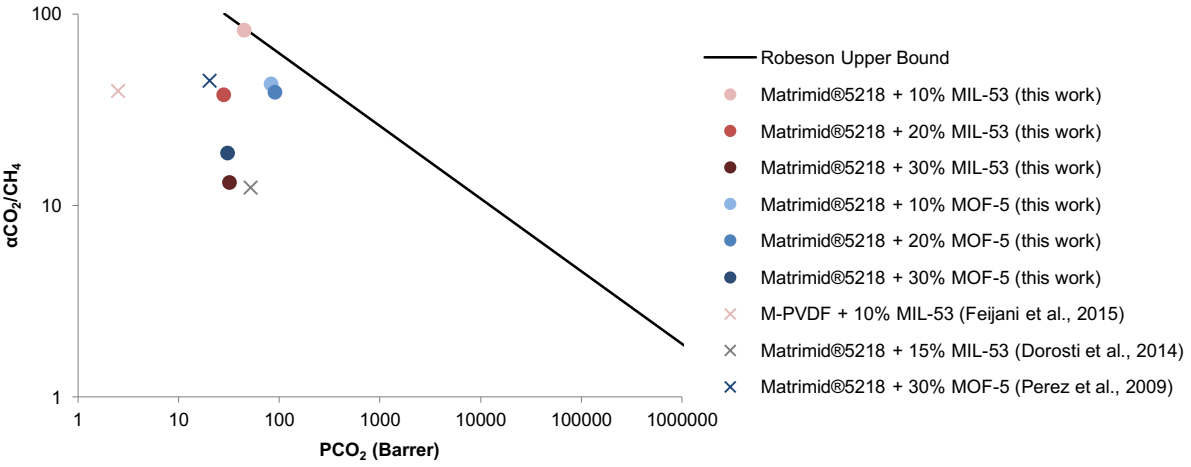

Figure 3.20 Robeson plot for CO_2/CH_4 with the results from the current and previous studies.

4

Conclusions

To the best of our knowledge, this is the first time different monosubstrates, in this case, fruit pulp wastes, were treated sequentially in the same operation in an anaerobic digestion system. The effect of this type of substrate shifts on the system performance was assessed in this study. As the ultimate goal is the industrial implementation of the treatment system, which would contribute for the decentralised management of wastes, it is of great importance to study the robustness of the system treating seasonal wastes. The two-stage anaerobic digestion system showed to be efficient in the treatment of seasonal wastes of a fruit juicy industry. The acidogenic and methanogenic reactors were operated for 285 and 178 days, respectively, without reactor failure or long-term instability after being subjected to substrate shifts while maintaining the same operational conditions. Moreover, the system recovered rapidly after stress situations such as electricity failure. Thus, it is reasonable to consider the full-scale industrial implementation of such a system which would be able to treat the diversity of wastes produced while providing an extra energy source for the company.

The fermentation of different substrates resulted in different gas productions in the acidogenic phase while slight differences were observed in FP concentration and profiles. HRT/OLR and pH changes did not seem to affect FP concentration and profiles in the acidogenic phase. High sugar removal was obtained (93.8 – 97.8%) as well as high acidification degrees (53.7% – 76.4%).

The study showed that the substrates used were highly biodegradable yielding high COD removal efficiencies in the methanogenic reactor (82.1 – 93.2%). Biogas production (3.6 – 12.8 L d^{-1}) and CH_4 productivity (0.54 – 2.13 L CH_4 L d^{-1}) increased through OLR increments up to 7.4 g COD L^{-1} (HRT 2.5 d) while CH_4 yield was fairly constant (0.30 – 0.37 L CH_4 g^{-1} COD). The high CH_4 content (76 – 81%) in the biogas was probably facilitated by the operation of a two-stage system. The CH_4 produced in the methanogenic reactor could generate energy up to 432.8 kJ d^{-1}.

The MMMs tested in this study showed potential to be used for the separation of CH_4 and CO_2. Membranes with 30% (w/w) MOF loading seemed to have poorer performances when compared with to 10 and 20% (w/w) loadings. Matrimid®5218 with 10% (w/w) MIL-53 membrane presented the best performance among the membranes tested and when compared with literature.

5

Future work

One crucial step for the implantation of this treatment system in full-scale is the cost reduction in terms of, for instance, alkali control and nutrients addition. In order to save the resources used on alkaline pH control and to reduce the amount of methanogenic effluent to be discharged, it would be interesting to study the use of the methanogenic effluent to dilute the substrate of the acidogenic reactor (Ganesh et al., 2014 and references therein). Moreover, the optimisation of the ratio of nutrients to be added to the influent could also be performed.

In order to assess the methanogenic reactor capacity to treat high organic loadings, it would be interesting to test even lower HRTs and higher OLRs. This study could be useful to maximise the amount of waste treated in a period of time.

In the future, it would be necessary to perform further membrane characterisation studies. These would include: (1) the evaluation of mechanical properties such as the puncture stress and elongation at break by puncture tests and (2) the evaluation of surface properties by SEM. Furthermore, mixed gas permeation studies using CH_4/CO_2 binary mixtures in different ratios (e.g. 60/40 and 90/10) would also be performed.

The implementation of a membrane upgrading system on-site could be an important goal. In order to minimise CO_2 release, it would be interesting to investigate the recirculation of CO_2 to the first reactor as it had been reported that the injection of CO_2 can increase HAc and overall VFA production as well as H_2 production (Fernández et al., 2015; Liu et al., 2017; Salomoni et al., 2011). Alternatively, the photosynthetic CO_2 uptake by microalgae may also be an interesting route to explore (Yan et al., 2016; Zhou et al., 2017).

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